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Effectiveness of Varenicline vs. Varenicline plus Bupropion or Placebo for Smoking Cessation

PI: Paul M. Cinciripini, Ph.D.

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Overview

It is estimated that cigarette smoking is responsible for over 40% of premature deaths and disability in the US. The adverse health risks of smoking increase significantly with duration and amount smoked per day, and it is precisely these heavier and more nicotine dependent smokers who are most refractory to treatment [1]. Although significant advances have occurred in smoking cessation therapy, approximately 23.4% of men and 18.5% of women continue to smoke [2]. Smoking has been characterized as a chronic relapsing disorder. Over 40% of smokers make a serious cessation attempt each year but less than 3% of all smokers successfully quit [3].

Nicotine is a ubiquitous drug with subtle effects on a smokers' mood and cognitive performance. Difficulty quitting has been strongly related to affective and cognitive dysfunction following abstinence. Multiple biological pathways (i.e., dopaminergic, noradrenergic, cholinergic) and structures (i.e. limbic system-prefrontal cortex and Anterior Cingulate Cortex-ACC) are involved in mediating these effects and hence pharmacologically based treatments have focused on several biological targets. While current smoking cessation pharmacotherapies have produced several positive outcomes [4–6], no "magic bullet" has emerged, nor does it seem likely that will be the case. Much like the treatment of depression, the psychobiological complexity of the nicotine dependence disorder suggests that multiple therapies should be made available to account for individual differences in treatment response.

The focus of this proposal is the evaluation of the combined effects of varenicline and bupropion, both in their own right FDA approved medications for smoking cessation. Both have shown effectiveness in randomized clinical trials for smoking cessation and the treatment of nicotine withdrawal symptoms. Bupropion is an atypical antidepressant whose properties include inhibition of norepinephrine re-uptake, modest dopamine re-uptake inhibition and noncompetitive nicotine antagonist effect [7]. Vann [8] have found it to exhibit both NACHR agonist and antagonist-like effects.

Varenicline acts primarily as a strong and highly selective partial agonist of the α4β2 nicotine cholinergic receptor in particular at the Ventral Tegmental Area (VTA) of the mesolimbic dopamine system, resulting in an attenuated release of dopamine in the nucleus accumbens, relative to nicotine. The results from the pivotal trials of varenicline showed it to be more effective than bupropion alone. However, there are several lines of reasoning to suggest that the combination of these drugs might be more effective than varenicline used in isolation. Plausible differences in the mechanisms of action of the two drugs and differences in their intensity of action within the same pathways, suggest that combining these medications will affect a broader range of biological targets identified as important for smoking cessation. For example, one might expect enhanced effects on the dopaminergic pathway by combining the two drugs: reduced re-uptake of dopamine as a function of bupropion; and a more sustained release of dopamine in the nucleus accumbens with varenicline (albeit attenuated relative to nicotine). Varenicline has not been shown to have noradrenergic properties and is unlikely to have strong antidepressant effects (recent reports suggest it may have depressogenic effects in some individuals), whereas bupropion does affect the noradrenergic pathways which are believed to account in part for its antidepressant effects. This may offer an additional advantage of the combination therapy. At least one study has suggested that bupropion may improve the abstinence rates among those with a history of depression, relative to placebo. It may be that those with a history of depression are more likely to experience an exacerbation of symptoms while quitting and are possibly at greater risk for this the varenicline group. The addition of bupropion might reduce the likelihood of this occurring. In addition, while both medications have been shown to reduce nicotine withdrawal, negative affect, craving and some indices of smoking reinforcement (satisfaction), some of the subscales of these measures are affected by one drug and not the other and varying effect sizes between the two drugs are observed for the others. Moreover, the separate and combined effect of both drugs on noradrenergic and dopaminergic pathways involved in cognitive and attentional function might ameliorate deficits in these areas related to smoking cessation. This raises the possibility that additive effects on several psychological predictors of relapse (e.g., negative affect and craving) as well measures of smoking reinforcement and cognitive performance might be observed when the two drugs are combined.

The vast majority of research in the pharmacological treatment of nicotine dependence has focused on the evaluation of single medications in placebo controlled trials, which by definition represents a "one-size fits all" approach to the application of the treatment. While this is appropriate in the initial stages of treatment

evaluation, the potential for combined treatments to raise the level of efficacy above individual treatments is often unexplored. Medication development research is almost exclusively sponsored by pharmaceutical companies, whose interests are not typically served by taking a combination approach (particularly when the other medication is generic or made by another company, although there are exceptions). It remains for researchers outside the pharmaceutical industry to evaluate such treatments. While it is still early in the history of use of varenicline, it appears to represent a significant advance over the treatments available to date. Nevertheless, the potential for improving treatment efficacy does exist, and combination therapies represent one way in which this may be realized. Increasing the diversity of available treatments for nicotine dependence can have a significant impact on the public health, as we attempt to understand, and in some cases reduce the impact of individual differences on treatment response. However, the first step in this process is to establish the efficacy of a treatment (combined) so that it may enter the mainstream of available therapies and that is the main objective of this proposal.

A. Specific Aims

Primary Aim:

1. To evaluate the efficacy of varenicline plus bupropion (VB) vs. varenicline (V) or Placebo (P) alone for smoking cessation,
   1.1 We hypothesize that smokers treated with the combination therapy will be abstinent significantly more often and take a longer time to relapse at 12 months follow-up than those treated with either varenicline or placebo alone.

Exploratory Aims:

1. To evaluate the effects of VB vs. V and P on measures of nicotine withdrawal, negative affect, smoking reinforcement, sleep problems, and craving, and measures of cognitive performance.
   1.1 We hypothesize that smokers treated with the combination therapy will report significantly lower levels of nicotine withdrawal symptoms, negative affect, depressive symptoms, smoking reinforcement, sleep problems, and craving over the course of treatment, and improved cognitive performance during quitting, than those treated with varenicline or placebo alone.
2. To evaluate the effects of VB vs. V and P on lapse progression
   2.1 This is an exploratory aim to evaluate possible differences between the two active treatments on time to an initial lapse; and time between an initial lapse and relapse. This later analysis will involve smokers who achieve initial abstinence but who are not continuously abstinent during treatment. This is a group of smokers for which little is known given the concentration of pharmaceutical research on treatment efficacy involving smokers who are continuously abstinent. We will test for the possibility that VB is more beneficial than V or P on preventing a lapse from resulting in a relapse.
3. To evaluate the effects of VB vs. V and P on smoking reduction among those who fail to quit.
   3.1 We hypothesize that non-abstinent smokers treated with VB will smoke significantly less than smokers treated with V or P.

B. Background and Significance

In the following sections, we will review converging lines of evidence suggesting that VB may be an important combination treatment for smoking cessation pharmacotherapy. The target population for this study is the general population of smokers. We will review current drug treatments for smoking cessation their pharmacological properties, and provide a rationale for the clinical use VB in the treatment of nicotine dependence.

Pharmacological Treatment used for Smoking Cessation. Until just recently, much of the focus of pharmacotherapy for smoking cessation has been on the use of nicotine replacement therapies (nicotine gum, patch, and lozenge) and antidepressants for the treatment of nicotine dependence. Several studies and meta-analyses have established the efficacy of the nicotine replacement therapies in smoking cessation, showing approximately a doubling of cessation rates over placebo [9]. Although numerous antidepressants have been

examined in clinical trials for their effectiveness to treat smoking cessation, only bupropion and nortriptyline have been consistently related to long term abstinence from smoking [10].

Bupropion (amfebutamone) is an atypical antidepressant whose mechanism of action is thought to be mediated through both dopaminergic (DA) and noradrenergic (NE) systems. Findings from in vitro experiments suggest that bupropion is a modest inhibitor of NE uptake and weaker inhibitor of DA uptake [11]. Bupropion produces a dose-dependent increase in dopamine concentrations in the nucleus accumbens in the mesolimbic system of rats [12]. Other research suggested that bupropion may also act as a noncompetitive nicotine antagonist, which might reduce the reinforcing properties of nicotine.[13] In particular one of its metabolites' (2S,3S) hydroxybupropion has been postulated as a better antagonist on $\alpha^4\beta^2$ nicotine receptor target than bupropion itself [14].It is of interest that Vann [8] have found bupropion to exhibit both nACHR agonist and antagonist-like effects in presence of nicotine, and if confirmed this would be similar to a partial agonist effect seen with varenicline, which could lead to possible potentiation when the two medications are combined. Studies examining bupropion's antidepressant effects suggest it is more effective in suppressing the firing rate of norepinephrine than dopamine neurons at locus coeruleus (LC) in rats [15,16]. A decrease in the neuronal firing rate in the LC is an indication of high level of synaptic norepinephrine concentrations possibly caused by a blockade of neurotransmitter reuptake. Thus, it is suggested that bupropion's antidepressant effects may be mediated through noradrenergic rather than dopaminergic pathways. Interestingly, desensitization of α^2 adrenoreceptors [17] is also thought to play a role in the negative affect associated with cocaine withdrawal, a situation like that of nicotine withdrawal, that enhances relapse vulnerability, In a meta-analysis based on 31 clinical trials of bupropion administered to over 10,000 participants, Hughes and colleagues [10] found that smokers who received the drug were twice as likely as those who received placebo to have achieved long-term abstinence (OR= 1.94; 95% CI, 1.72 -2.19,). At least one study suggests negative affect reduction as measured by the PANAS (Positive and Negative Affect Scale[18] may mediate bupropion's efficacy [19].

Nortriptyline is a tricyclic antidepressant that has highly specific effects on NE reuptake inhibition [20]. It is10 times more potent than venlafaxine (a drug used in our previous studies) in this regard and about 7 times less effective at serotonin reuptake inhibition. In contrast to bupropion, it is almost 50 times more effective at NE reuptake inhibition but 6 times less effective at dopamine reuptake inhibition (in vitro) [20]. In the most recent Clinical Practice Guidelines, nortriptyline has been recommended as an effective treatment for smoking cessation [21]. While seemingly effective, nortriptyline is typically less well tolerated than bupropion and is not typically used in everyday treatment of nicotine dependence. Bupropion in contrast to nortriptyline is an FDA approved medication for smoking cessation, has far fewer side effects, and requires much less medication management and pretreatment screening in clinical practice. For example, prior to nortriptyline therapy physicians often conduct a screening ECG to rule out AV block or arrhythmias. Blood levels are also monitored after therapy is initiated in order to titrate the dose to a therapeutic range. Neither medical management procedures are routinely conducted for either bupropion or varenicline.

Other antidepressants including doxepin, fluoxetine, imipramine, moclobemide, paroxetine, sertraline and tryptophan, and the anxiolytic, buspirone have also been examined in smoking cessation trials. While some individual studies have shown short term or limited success with these drugs, most have failed to support a significant effect on long-term abstinence [22]. The exception is our study using venlafaxine, a norepinephrine and serotonin reuptake inhibitor that was found to improve the chance of abstinence among smokers who smoked less than a pack of cigarettes per day[23].

In the summer or 2006, a new agent, varenicline (Chantix ®) was approved by the FDA for smoking cessation. Varenicline is a highly selective partial agonist of the $\alpha^4\beta^2$ nicotine cholinergic receptor (nACHR). It stimulates dopamine release in the nucleus accumbens (nAC) of animals, but to a much less extent than nicotine itself, and by binding at the receptor throughout its relatively long half-life (24 hrs), it displays antagonist properties, by preventing full stimulation of the receptor that ensues when nicotine is co-administered[24]. Thus varenicline has the potential to provide relief from withdrawal (agonist effect) and block the rewarding effects of nicotine (antagonist effect) [25]. In addition to its effects at the $\alpha^4\beta^2$ nACHR, animal studies have also shown that varenicline acts as a full agonist of the α^7 nACHR [26]. This has interesting implications for the drug that are not typically discussed when considering its mechanism of action. For example, presynaptic α^7 nACHR's enhance glutamatergic excitatory drive whereas active $\alpha^4\beta^2$ nACHR's directly excite DA neurons. The additive effects of presynaptic glutamate release and postsynaptic firing

increases the likelihood of synaptic potentiation (e.g., Long Term Potentiation). In addition, $\alpha^4\beta^2$ nACHR's are found on the cell body of GABAergic interneurons that project to DA neurons in the VTA. Prolonged nicotine exposure causes some desensitization of these receptors; eventually decreasing their inhibition of VTA DA neurons, resulting in a complex interplay between these neural connections that acts to facilitate DA release in the nAC, even as $\alpha^4\beta^2$ nACHR receptors on DA cell bodies are desensitized [27]. While it is unknown whether or not varenicline may act in a similar fashion, the possibility exists that its effects on the α^7 glutamatergic and $\alpha^4\beta^2$ GABAergic nACHR receptors result in a similar pattern of DA stimulation, hence providing several routes of action to facilitate smoking cessation.

Two randomized, double-blind clinical trials that compared varenicline (2 mg), bupropion (300 mg), and placebo showed an overall continuous abstinence rates between the end of treatment through the one year follow-up of 21.9%, 16.1%, and 8.4%, respectively in one study [28] and 23%, 14.6% and 10.3%, respectively in another[6]. All comparisons were significantly different although the alpha level of .057 was observed in the bupropion vs. varenicline comparison in the Gonzales trial. In a combined analysis of both trials[28] varenicline resulted in significantly higher continuous abstinence rates at one year compared to either placebo or bupropion (all p values were <.05). In these studies, varenicline nearly tripled the odds of quitting over placebo during the last 4 weeks of treatment (OR, 3.09; 95% CI, 1.95-4.91; P<.001)[28]; (OR, 2.66; 95% CI, 1.72-4.11; P<.001) [6]. Moreover, in a separate study, an additional 12 weeks of varenicline therapy (total of 24 weeks) has been shown to reduce the risk of relapse among smokers who were abstinent at the end of the first 12 weeks[29]. Compared to smokers who received placebo, those who received varenicline reported significantly less craving and withdrawal symptoms throughout the trials [30]. Side effects of varenicline may include nausea and abnormal dreams (see Human Subjects for listing of AE's).

Rationale for combining therapies. The focus of this proposal is the evaluation of the combined effects of varenicline and bupropion, both in their own right FDA approved medications for smoking cessation. As discussed above, plausible differences in the mechanisms of action of the two drugs and differences in their intensity of action within the same pathways, suggest that combining these medications will affect a broader range of the biological targets identified as important for smoking cessation. For example, considering bupropion's effects on noradrenergic and dopaminergic reuptake inhibition, one might expect a synergistic effect with the addition of varenicline: reduced re-uptake of dopamine as a function of bupropion; a more sustained release of dopamine in the nucleus accumbens with varenicline; and/or a stabilization of the noradrenergic pathways which could lead to reduced vulnerability to negative affect while quitting. Varenicline has not been shown to have noradrenergic properties and is unlikely to have strong antidepressant effects, whereas bupropion does affect the noradrenergic pathways which are believed to account in part for its antidepressant affects. Relative to placebo, bupropion may improve the abstinence rates among those with a history of depression [31], a condition one might consider a risk factor for negative affect while quitting, placing such individuals at a relatively greater risk of relapse (findings are not uniform regarding the inverse relationship of quitting with or without bupropion to history of major depression [10]). Both bupropion and varenicline have been shown to reduce nicotine withdrawal, negative affect, craving, and some indices of smoking reinforcement (satisfaction). However, relative to placebo, varenicline shows greater effect sizes for reducing smoking satisfaction, psychological reward, enjoyment of respiratory sensations smoking urges, negative affect, restlessness, and craving [4]. Interestingly, varenicline actually shows an increase in appetite relative to placebo, a fact that is likely explained by the relatively greater proportion of abstainers in the varenicline group. Nevertheless, the same is not true for bupropion which is known for producing modest appetite suppression. Bupropion has been shown to offset the deficits in cognitive and attentional functioning related to smoking cessation. This raises the possibility that additive effects on several psychological predictors of relapse (e.g., negative affect and craving) as well measures of smoking reinforcement and cognitive performance might be observed when the two drugs are combined. In addition, among those smokers for whom the possibility of weight gain might adversely affect their treatment success; the addition of bupropion could have a beneficial effect.

Considering bupropion's recently noted nACHR antagonistic effects it is possible that the additional agonistic effects of varenicline would be enhanced within such an antagonist rich environment. Partial agonists are known to behave like agonists in an antagonist rich environment (bupropion in this case). An example of this type of interaction also comes from studies combining mecamylamine (nicotine antagonist)

with the nicotine patch (agonist) [32], where favorable short term effects have been noted for smoking cessation. Unpublished data has not supported long term effects of this combination (E.D Glover, personal communication). Similarly, previous trials combining the nicotine patch with bupropion have also shown a significant enhancement of abstinence rates with the combination in the short term, and although not statistically significant, the combined approach also produced elevated abstinence rates at long term follow-up [33]. In the mecamylamine and bupropion studies, nicotine, while itself a full nACHR agonist, can be expected to have much more limited agonist effects when administered in patch form, given the distribution kinetics of the patch, relative to the targeted and longer lasting agonist properties of varenicline. From another point of view, it may also be noted that partial agonists (i.e., varenicline), in an agonist rich environment (i.e., smoking during a lapse) is expected to behave like antagonists having occupied receptor sites. Thus nACHR antagonism may be enhanced by the two drug combination, providing a particularly supportive environment for reducing the reinforcing effects of nicotine during lapse related smoking, which ultimately should reduce the probability of a lapse resulting in a complete return to smoking. In both scenarios (agonist/antagonist rich environment), the combination of both varenicline and bupropion may provide stronger and wider reaching agonist and antagonistic effects on effects nACHR's, which may translate into improved rates of cessation over varenicline alone.

Smoking and Psychiatric Co-morbidities**.** Smoking is often co-morbid with substance, mood, and attention disorders suggesting shared biological pathways between nicotine dependence and these psychiatric conditions. While a complete review of this area is beyond the scope of this proposal, suffice it say that several studies have demonstrated a positive relationship between alcohol, substance abuse and other psychiatric disorders and smoking [34–37;38–41]. For example, the lifetime prevalence rate of alcohol dependence or drug abuse is estimated at 23%-30% among adult smokers [42,43]. Among non-dependent and dependent current smokers lifetime rates of mood and anxiety disorders have been reported as 12%-26.7%, and 33.5%-46.5%, respectively [43]. Similarly, as shown in **Figure 1**, among tobacco dependent smokers, 12-month prevalence of any mood or anxiety disorder was 21%-22%, respectively [44]. There is also an elevated risk of first onset of major depression, panic disorder, and generalized anxiety disorder among smokers [45–47]. In the area of cognitive dysfunction, odds ratios comparing ever to never smokers were positively related to the number of Attention Deficit Hyperactivity Disorder (ADHD) symptoms; and among those reporting regular smoking over their lifetime, an inverse relationship between number of (ADHD) symptoms and age of onset, and positive relationship between symptoms and number of cigarettes smoked, has also been observed [48].

Figure 1. 12 Month Prevalence of Comorbid Disorders & Nicotine Dependence

Comorbid Disorder	12-Month Prevalence of Comorbid Disorder Among Respondents With Nicotine Dependence, % (SE)	12-Month Prevalence of Nicotine Dependence Among Respondents With Comorbid Disorder, % (SE)	Odds Ratio* of Nicotine Dependence and Comorbid Disorder (95% Confidence Interval)
Any alcohol use disorder	22.8 (0.72)	34.5 (1.11)	4.4 (3.9-4.9)
Alcohol abuse	9.3 (0.52)	25.5 (1.31)	2.5 (2.1-2.9)
Alcohol dependence	13.5 (0.61)	45.4 (1.77)	6.4 (5.6-7.4)
Any drug use disorder	8.2 (0.49)	52.4 (2.29)	8.1 (6.7-9.8)
Any drug abuse	4.8 (0.41)	44.7 (2.86)	5.7 (4.5-7.3)
Any drug dependence	3.4 (0.31)	69.3 (3.46)	15.9 (11.4-22.2)
Any mood disorder	21.1 (0.73)	29.2 (1.03)	3.3 (3.0-3.6)
Major depression	16.6 (0.63)	30.0 (1.07)	3.3 (3.0-3.7)
Dysthymia	4.6 (0.35)	32.0 (2.08)	3.3 (2.8-4.0)
Mania	4.6 (0.34)	35.3 (2.32)	3.9 (3.2-4.7)
Hypomania	3.0 (0.31)	33.4 (2.82)	3.5 (2.7-4.5)
Any anxiety disorder	22.0 (0.78)	25.3 (0.82)	2.7 (2.4-3.0)
Panic disorder with agoraphobia	1.8 (0.22)	39.8 (3.52)	4.6 (3.4-6.2)
Panic disorder without agoraphobia	4.3 (0.36)	35.6 (2.57)	3.9 (3.2-4.8)
Social phobia	5.8 (0.42)	27.1 (1.67)	2.6 (2.2-3.1)
Specific phobia	14.3 (0.63)	25.6 (0.95)	2.6 (2.3-2.9)
Generalized anxiety	5.3 (0.41)	32.7 (2.02)	3.4 (2.8-4.2)

Although other antidepressants and anxiolytics have not generally been found efficacious for smoking cessation [10], bupropion's antidepressant actions may make it a particularly attractive choice for smokers vulnerable to negative affect or among those with some level of affective and/or cognitive impairment. For example, in one study relative to placebo, bupropion was shown to be effective among smokers with a history of depression [31], although absolute cessation rates among depression history positive ad negative smokers may not differ[10]. Bupropion has also been used in a preliminary study to treat smokers with PTSD [49]. Apart from smoking, bupropion has long been indicated for the treatment of depression, and recent studies have shown additional benefits including prevention of the recurrence and improved efficacy for depressed patients

with concomitant anxiety (see Clayton [50]), as well as a favorable outcome for the treatment of cocaine addiction when combined with behavioral treatment [51]. Direct tests of bupropion with depressed smokers have not been carried out since those with concurrent depression are typically excluded from smoking cessation pharmaceutical trials. Similarly, given concerns about bupropion's seizure potential during alcohol withdrawal, it has not been directly used in the treatment of smokers with alcohol use disorders (although other antidepressants have with similar noradrenergic properties have shown efficacy in the treatment of depressed alcoholics (see Torrens [52]). We have noted no untoward safety concerns in an open label trial of bupropion with alcoholic smokers [53]. Nevertheless, the potential remains for bupropion to have a favorable impact on smokers with some level psychiatric symptoms. The combination of these medications may have particular importance since recent post-marketing reports that varenicline may be associated with increased depressive symptoms in some individuals (i.e., those with a history of depression). Bupropion might reduce the likelihood of this occurring**.** It is plausible that in a community sample such as ours with a wide range of sub-threshold disorders, bupropion could exert a favorable impact on such individuals attempting to quit smoking. When combined with varenicline, treatment efficacy is likely to improve, since both the psychiatric symptoms as well as the direct agonistic effects on nACHR's receptors are addressed,

Bupropion's role in the area of cognitive dysfunction represents another important area of possible synergy with varenicline. Difficulty concentrating is a symptom of the tobacco withdrawal syndrome [54]. In addition, acute abstinence from tobacco generally leads to decrements on cognitive tasks[55,56] including attention tasks [57]. Studies have documented that bupropion has beneficial effects on questionnaire measures of attention. Gobbi and colleagues [58] administered 0 mg, 150 mg, or 300 mg bupropion to 24 healthy male volunteers. Participants on 300 mg bupropion reported improved self-reported attention. Wilens [59] administered 300 mg bupropion to 36 adult ADHD and/or bipolar disorder. Participants reported reduced distractibility and improved concentration at 6 weeks. Some studies have reported that bupropion can improve performance on objective cognitive assessments. Shiffman [60] reported that 300 mg (but not 150 mg) bupropion significantly speeded reaction times on a logical reasoning task (vs. placebo), and marginally improved error rates. Evins and colleagues [61,62] administered up to 300 mg bupropion to 52 adult schizophrenics. Participants on bupropion exhibited reduced preservative errors on the California Verbal Learning Test, and reduced variability of reaction times on the Conners Continuous Performance Task (a test of sustained attention).

Bupropion has also been shown to be effective in the treatment of adult ADHD [63]. The overlap between symptoms of ADHD and smoking prevalence has already been discussed above. There may also be implications for treatment of ADHD symptoms and smoking cessation success, for which bupropion might have an ameliorative effect. For example the quit ratio among ever smokers is lower (29%) among smokers with a history of ADHD as compared to the general population (48%) [64]. Similarly, a history of childhood ADHD in adults, has been shown to predict time to relapse in a study where only 1/47 such smokers remained abstinent at a one year follow-up (OR-.36 CI .28-.45) [65]. However, at least one study suggests that when smokers are treated for ADHD, both ADHD symptoms and frequency of smoking decrease [66]. Conversely, an increase in the number of ADHD symptoms during the early phases of a quit attempt also predicts relapse to smoking [67]. In addition, among smokers with current ADHD symptoms of inattention, a primary reason cited for smoking is to control negative affect, which itself is a robust predictor of relapse [68]. Adding varenicline to the mix may have additional benefit. For example, in laboratory settings, nicotine and nicotine agonists have been shown to improve attentiveness and cognitive function [69], increase memory [70], and significantly reduce other symptoms associated with ADHD [71,72]. Taken together, these studies suggest that the combination of bupropion and varenicline might be particularly effective in reducing symptoms of cognitive dysfunction that might occur during a cessation attempt and possibly adversely affect the smoker's chances of cessation success.

Biological Pathways in Nicotine Dependence. Nicotine is the primary psychoactive substance in tobacco smoke and has diverse effects throughout the CNS. It modulates the activity of several neurotransmitters including, dopamine (DA), norepinephrine (NE), serotonin (5-HT), glutamate (GLUT), and γ-aminobutyric acid (GABA), as well as endogenous opioid peptides by binding to nicotinic cholinergic receptors (nAChr) throughout these neuroregulatory systems. While much of the focus of previous research on nicotine addiction has been related to its effects on reward processes and mesolimbic dopamine neurotransmission [73–75], and will not be reviewed here, a growing body of literature suggests that it's noradrenergic (and dopaminergic) effects on attention, information processing and affective regulation, elsewhere in the limbic

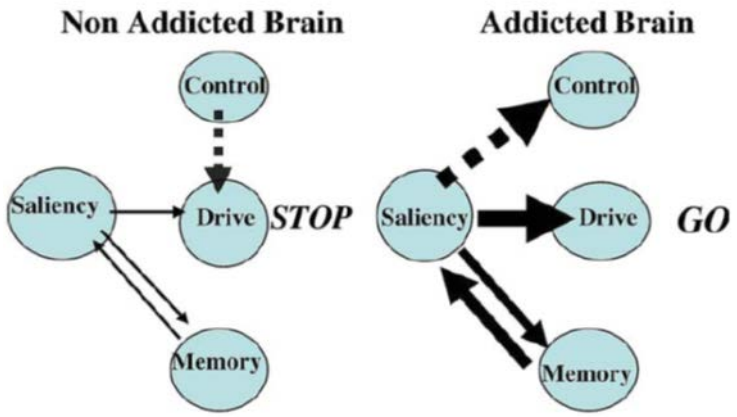
system, may be of considerable importance in understanding the maintenance of dependence. These effects are particularly relevant to understanding how varenicline and bupropion might complement each, through shared biological mechanisms between nicotine dependence, attentional disorders, other addictions, and mood and anxiety comorbidities discussed above.

Neurological deficits common to attention and substance use disorders, such as impaired performance, lack of motivation, decreased working memory and impaired executive function have been well documented [76] in both children and adults [77–81]. Current lines of investigation suggest that overlapping interrelated brain areas are responsible for explaining the attentional and executive impairments common to the two disorders [82,83]. The involvement of two areas in particular, prefrontal cortex (PFC) and anterior cingulate cortex (ACC) highlight the commonalities between drug dependence, including nicotine and neurophysiological deficits related to cognitive dysfunction.

The PFC regulates goal directed behavior, thought, and affect by using working memory to provide representational knowledge about past or future events and integrating this information into a plan for action or to exercise inhibitory control over inappropriate actions or thoughts. In attentional/cognitive disorders these processes are impaired and manifested in symptoms that involve poor attention, planning, impulse control, and monitoring of one's behavior. Studies indicate that the right PFC in humans is particularly important in the inhibition of activity (i.e., Stop or Go-No Go tasks)[84]. The orbital and ventral PFC may also have a similar inhibitory effect in the affective domain, thus permitting appropriate social behaviors (e.g., Anderson et al. [85],and Stuss [86]. In ADHD for example, the ACC has also been implicated in the regulation of the motivational aspects of attention as well as in the regulation of response selection and inhibition [76]. Thus, researchers have begun to characterize ADHD as a disorder with deficits in inhibitory processes involving frontal cortical structures[77]. If a person must mentally manipulate information and make a response, the anterior cingulate (with its connections to the PFC) becomes active [87]. This area become particularly active in tasks where inhibitory control or divided attention are necessary [88].

The importance of the inhibitory role of these structures in drug dependence has also been highlighted by several researchers. Drug addicted individuals, including smokers, continue to use drugs even when faced with negative consequences and diminished reward, suggesting an apparent loss of control [89]. The failure to regulate (inhibit) this drive points to a dysfunction within the PFC [90] and related areas including the anterior cingulate and orbitofrontal corticies [91]. As shown in **Figure 2,** the resulting persistence of the behavior is not necessarily due to continued reinforcement by the drug (mesolimbic dopamine) but rather to the enhanced saliency of the drug and drug cues that have been firmly established (learned) in memory during the acquisition of dependence. During maintenance of drug dependence these "super salient" drug related cues, including self-administration, overcome the inhibitory control of the PFC that might normally extinguish a response with decreasing hedonistic properties. Preclinical studies suggest that the impairment in PFC function may be related to significant dendritic branching and spine density resulting from repeated drug administration [92], thus amplifying the signal of salient events. Moreover, abstinence, for example from smoking, significantly reduces the efficiency of the PFC to process information in working memory thereby interfering with its regulatory function[93]. Such effects might be mediated by the negative affect associated with nicotine withdrawal, and when present, reduce the probability that a smoker may exercise an appropriate coping response and increase the probability of relapse [94,93]. There is EEG evidence supporting persistent frontal lobe dysfunction among smokers using tasks related to working memory (P300). Neuhaus and colleagues [95] found a hypoactivation of the anterior cingulate, orbitofrontal, and prefrontal cortex among both current and former smokers compared to never smokers, suggesting that the dysfunctional activation patterns found in smokers may not completely remit after quitting; a fact that may increase their vulnerability to relapse. Bupropion may reduce this deficit. For example, in a recent imaging study bupropion treated smokers had smaller cigarette cue induced increases in craving scores and less activation of perigenualyventral ACC (which projects to the PFC), relative to neutral cues than did untreated smokers [96].

Figure 2.



Addiction model proposed based on imaging findings documenting abnormalities in brain circuits involving saliency/reward, motivation/drive, memory/conditioning, and control/disinhibition. These circuits interact with one another and change as a function of experience and context. During addiction, the enhanced saliency value of the drug in the reward, motivation, and memory circuits overcomes the inhibitory control exerted by the prefrontal cortex. A positive feedback loop initiated by consumption of the drug and perpetuated by the enhanced activation of the motivation/drive and memory circuits results in compulsive drug seeking and taking. Taken from Volkow (Volkow et al., 2004)).

Perhaps, the combination of varenicline with its direct effects on the VTA, DA release in the nAC and associated smoking related reward on the one hand, and the diverse effects of bupropion on central NE function with noted ameliorative effects on cognitive function, on the other, may make a particularly powerful drug combination for smoking cessation. Each drug adds to the diversity of biological targets which may play a role in the maintenance of smoking behavior.

Norepinephrine and Dopamine. The above pathways are rich in catecholamines. Cognitive symptoms related to ADHD for example, are relieved by blocking DA and NE reuptake into the presynaptic neuron, thereby increasing the concentration of these neurotransmitters in the extracellular space [97]. It appears that DA dysfunction in the lower striatal areas reduces attention, focus, acquisition, and cognition, whereas NE dysfunction in the PFC is associated with distractibility, poor executive operations, and reduced behavioral and cognitive inhibition [98]. While imaging studies are required to verify the scope of varenicline's anatomical targets, it is indeed plausible that varenicline acts on the DA projections from the nAC to the PFC and in the lower striatum in a manner consistent with that of nicotine [99], which itself has been shown to improve attention and memory function (see Levin et al for review [100]).

NE mechanisms have not been a major focus in drug abuse, however, NE is able to modulate midbrain DA function important to drug reward [101]. Nicotine also releases NE in various CNS regions [102] via nicotinic-type acetylcholine receptors [103,104]. At least two lines of evidence point more directly to the role of NE in nicotine reinforcement. First, Reboxetine, a NE reuptake inhibitor attenuates nicotine self-administration [105]. Second, NE secretions in the paraventricular nucleus (PVN) increase during continuous nicotine self-administration [106]. NE mechanisms (desensitization of α^2 adrenoreceptors) [17] are also thought to play a role in the negative affect associated with cocaine withdrawal, a situation like that of nicotine withdrawal, that enhances relapse vulnerability, As discussed above, both bupropion and nortriptyline inhibit the reuptake of norepinephrine and both have been shown to be effective for smoking cessation. Taken together these studies suggest that a nicotine agonist like varenicline, could possibly add to the overall effects associated with bupropion elsewhere in the brain, to further offset cognitive deficits as well as the affective disturbances associated with quitting smoking that might adversely affect treatment success. The etiology of nicotine dependence and associated co-morbidities and the mechanism of action of shared pharmacotherapies suggest that regulation of NE and DA neurotransmission are involved in ameliorating affective and cognitive deficits associated with quitting.

Need for current research. As indicated above, current smoking cessation pharmacotherapies using NRT, bupropion, nortriptyline [10], and now varenilcine[28] have produced several positive outcomes. However no "magic bullet" has emerged, nor does it seem likely that will be the case. Much like the treatment of depression, the psychobiological complexity of the nicotine dependence disorder suggests that multiple therapies should be made available to account for individual differences in treatment response. Continuing to develop new treatments for nicotine dependence will have several significant benefits. First, new treatments

have the potential of having an immediate impact on public health and disease prevention, and in this case there may also be a benefit of both increased treatment efficacy and reduction in possible neuropsychiatric adverse effects of varenicline. Second, with an increasing diversity of available treatments, particularly combination therapies, future research may focus on tailoring treatments to particular characteristics of the individual (i.e. fitting a particular medication or treatment combination to a group of individuals based on genetic or other individual differences). Should this research prove successful, future proposals may focus on the discovering how such individual differences might map onto the complex biological response engendered by the combined treatment. For example, it is possible that the pharmacogenetic response of these drugs might be related to haplotypes of genetic polymorphisms of the NET (norepinephrine transporter gene), COMT (Catechol O-methyltransferase) and DRD2 -141 ins/del alleles. However, the first step in this process is to establish reasonable effectiveness of this combination treatment so that it may enter the mainstream of available therapies and that is the main objective of this proposal.

C. Preliminary Studies

We present a preliminary data from an open label study that was done with cancer patients who were referred to our tobacco treatment program. The Tobacco Treatment Program at the University of Texas M. D. Anderson Cancer Center is a large-scale comprehensive program that delivers tobacco-cessation counseling and medication to cancer patients. The patients present to the program with a wide variety of demographic, social, and economic backgrounds and cancer sites. The Tobacco Treatment Program has been in operation for little over 2 years. We do not have any exclusionary criteria for patients entering treatment. Patients in the program receive behavioral counseling from a clinician (Masters or Ph.D. level psychologist, APN) and a tobacco-cessation medication, which is managed by the program's medical staff led by Maher Karam-Hage, MD (co-investigator on this application and Associate Medical Director of the program). Most patients entering the program have had previous quit attempts that were unsuccessful.

Table 1 Demographics for Patients Selected for Preliminary Studies Sample

	Varinicline Only N = 63 Mean (*SD*)	Bupropion Only N = 16 Mean (*SD*)	Varinicline + Bupropion N = 11 Mean (*SD*)	Bupropion + Varinicline N = 7 Mean (*SD*)
Age	54.92 (11.94)	53.56 (8.79)	60.00 (7.55)	48.00 (8.62)
PANAS-Negative	19.71 (8.85)	19.80 (7.77)	21.50 (7.75)	24.86 (12.46)
PANAS-Positive	31.05 (8.85)	31.00 (10.78)	31.42 (9.52)	26.71 (6.53)
CES-D	14.07 (12.63)	14.13 (9.95)	14.00 (8.10)	22.29 (16.67)
PHQ Diagnoses	0.84 (1.00)	0.44 (0.63)	0.91 (0.83)	1.29 (1.38)
FTND*	4.09 (3.17)	5.44 (1.97)	6.18 (2.36)	3.83 (3.60)
Cigarettes Per Day	17.34 (15.31)	21.19 (11.37)	26.82 (11.89)	15.71 (15.25)
	n (%)	**n (%)**	**n (%)**	**n (%)**
Male	24 (38.1%)	11 (68.8%)	4 (36.4%)	2 (28.6%)
Female	39 (61.9%)	5 (31.3%)	3 (63.6%)	5 (71.4%)
White	51 (81.0%)	12 (75.0%)	11 (100%)	4 (57.1%)
Black	7 (11.1%)	2 (12.5%)	0 (0%%)	1 (14.3%)
Other	5 (8.0%)	2 (12.5%)	0 (0%)	2 (28.6%)

*** $p < .10$**

We present new pilot data below from an open label trial of treatment resistant smokers who are cancer patients/survivors referred to our clinical tobacco treatment program. This was not a randomized trial but rather a retrospective analysis of data from a clinical intervention program. For this analysis we first selected a group of smokers who had failed to quit after taking a single treatment agent (either varenicline or bupropion) for 8 weeks. In an attempt to help them quit the alterative medication was then added, while they continued taking the first one. Combining these two medications for treatment resistant patients is a relatively recent clinical intervention used in our clinic. Thus, for comparison we selected a similar group of patients (those failing to quit after 8 weeks of treatment) who were treated in close proximity to the time frame of those taking the drug combination. In total, there were 97 smokers in the sample: 63 taking varenicline alone (the most commonly prescribed medication in our clinic); 16 on bupropion alone; 11 who initially began varenicline for whom bupropion was added; and 7 who initially began bupropion for whom varenicline was added. The demographic characteristics of these groups are shown in **Table 1**. No significant difference between the groups was noted. However, smokers in the varenicline plus bupropion group (varenicline given first), did have

Table 2 Primary Cancer Type Diagnosis

	Varinicline Only N = 63	Bupropion Only N = 16	Varinicline + Bupropion N = 11	Bupropion + Varinicline N = 7
Breast	19 (30.2%)	3 (18.6%)	4 (36.4%)	1 (14.3%)
Gastrointestinal	16 (25.4%)	4 (25.0%)	0 (0%)	1 (14.3%)
Head & Neck/Thoracic	19 (30.2%)	5 (31.4%)	4 (36.4%)	4 (57.1%)
Other	9 (14.2%)	4 (25.0%%)	3 (27.2%)	1 (14.3%)

marginally higher FTND dependency scores than the other groups which could have had a deleterious effect on cessation from the start. The fact that we did improve abstinence rates in this group is impressive given their level of dependency and immediate history of lack of efficacy.

We also show no differences in primary cancer site among groups particularly for the Varenicline vs. Varenicline + Bupropion groups which are most germane to this proposal (see **Table 2**). In addition the medication combinations were well tolerated as we did not find evidence of a significant increase in common side effects or the emergence of any serious side effects when compared with those patients administered varenicline or bupropion alone, in this small sample (see **Table 3**).

Table 3 AE Profile Mono vs. Combined Therapies

	Varinicline Only N = 63	Bupropion Only N = 16	Varinicline + Bupropion N = 11	Bupropion + Varinicline N = 7
Oral	1 (1.6%)	1 (6.3%)	1 (9.1%)	1 (14.3%)
Gastrointestinal	9 (14.3%)	3 (18.8%)	4 (36.4%)	0 (0%)
Psychiatric	11 (17.5%)	1 (6.3%)	2 (18.2%)	0 (0%)
Neurologic	5 (7.9%)	0 (0%)	1 (9.1%)	0 (0%)
Cardiovascular**	0 (0%)	2 (12.5%)	0 (0%)	0 (0%)
Dermatologic	1 (1.6%)	0 (0%)	2 (18.2%)	0 (0%)
Other	6 (9.5%)	0 (0%)	1 (9.1%)	0 (0%)

** $p < .05$

Oral: Dry mouth, bleeding gums
GI: Nausea, constipation, gas
Psychiatric: Depression, nervousness
Neurologic: Dizziness, headache
Cardiovascular: Chest pain, shortness of breath
Dermatologic: Flushing, skin irritation
Other: Ear ache, feet swelling

Among those continuing on varenicline or bupropion alone, 7-day point prevalence abstinence rates at the end of treatment (12 weeks) was 14.3% (9/63) and 12.5% (2/16), respectively. Among those beginning on bupropion for whom varenicline was added and those beginning on varenicline for whom bupropion was added, abstinence rates 8 weeks after beginning the combination were 42.9% (3/7) and 27.3% (3/11). We chose the 8 week mark because this allows a comparable amount of exposure to the combination therapy that was allowed on the monotherapy before initiating the combination. Among this treatment resistant group of smokers, the data suggest that adding bupropion to varenicline results in nearly a doubling of abstinence rates: only 14.3% of these patients quit after receiving a full course on varenicline, while 27.3% quit on the combination. Though not of direct import to this study, the substantial increase in abstinence observed when varenicline is added to bupropion (42.9%) vs. bupropion alone (12.5%) is likely due to the fact that these smokers are treatment failures on bupropion, a less effective drug than varenicline to start with. When given the more effective medication their abstinence rates improve to a level similar to what has been observed at the end of treatment in the original clinical trials of varenicline (7-day point prevalence of 50%).

The most important comparison in this data for purposes of this proposal is the contrast between varenicline alone vs. varenicline plus bupropion, and our subsequent discussion is based on the possibility that the combination of varenicline plus bupropion is likely to result in improved efficacy over varenicline alone. The data shown here, while not a direct parallel of the clinical trial we propose to do, does suggest that the combination approach (varenicline + bupropion) may improve the efficacy rates among a group of treatment resistant smokers who would not have otherwise been able to quit (varenicline alone). The data do not tell us what the outcome would be if both medications were started in close proximity of one another (as proposed here); a scenario that might possibly produce stronger additive effects as discussed earlier. Starting both medications at the same time might result in “rescuing” more smokers, who may drop out early in the cessation process (prior to 8 weeks) due to lack of efficacy. Such smokers may become discouraged, and revert to smoking and become lost to follow-up. The favorable impact of a medication combination on these smokers early in the quitting process may be substantial if it brings about quitting sooner and improves the self-efficacy by facilitating a positive experience that would not have otherwise occurred.

There are of course caveats to be considered in this data, including the fact that it represents a self-selected sample of monotherapy non-responders. While the demographics and AE data may not differ across groups, it should be noted that the overall AE profile was lower than expected given the results of the pivotal studies. This sample is also comprised of cancer patients who may respond differently than our community sample, but it is worth noting that the 12 week abstinence rates for the over 300 patients in our program on varenicline is 48%, which is quite similar to the rates noted in the pivotal trials (44% continuous abstinence and 50% point prevalence). Nevertheless, these preliminary data provide a signal that the combination of varenicline and bupropion may improve abstinence rates over those of varenicline alone, and that a randomized clinical trial may be undertaken to investigate this further.

Experience of the PI. Paul M. Cinciripini, Ph.D. is Professor and Deputy Chair of the Department of Behavioral Science, and Director of the Tobacco Treatment Program, at the University of Texas MD Anderson Cancer Center. He has over 20 years experience conducting basic and clinical research in the area of smoking cessation and nicotine psychopharmacology. Dr. Cinciripini has conducted basic laboratory studies evaluating psychophysiological, psychopharmacological and genetic aspects of nicotine dependence. Examples of his work in this area include studies of nicotine titration and compensation, psychophysiological effects of nicotine during stress, individual differences in the effects of nicotine on EEG and cardiovascular activity, genetic factors treatment outcome, pharmacogenetic effects of antidepressants during smoking cessation, and recent studies using startle probe methodology to examine the relationship between genetics, emotional reactivity, nicotine exposure and nicotine withdrawal. He has also studied the influence of treatment process measures and psychological characteristics of the smoker on cessation success. Examples of his work in this area include studies of the effects of depression, coping behavior and self-efficacy as well as genetic factors related to nicotine dependence. In the area of clinical trials he has led studies testing of novel approaches for the treatment of nicotine dependence in the form of behavioral and pharmacological therapies used alone and in combination. Examples of his work in this area include: the development of a "scheduled smoking" procedure and a recent application of this technology for delivery on a handheld computer; development of a smoking cessation video series for pregnant smokers; evaluating combination therapies using nicotine replacement, behavioral counseling, and other approaches; and testing novel pharmacological compounds, including anxiolytics, antidepressants, nicotine partial agonists, and cannabinoid antagonists. Dr Cinciripini has been the recipient of several NIH, extramural and industry sponsored research grants and is the author of over 75 articles and book chapters.

He is currently PI on two NIH funded clinical trials. The major goal of the first project, "A Mood Management Intervention for Pregnant Smokers is to test the hypothesis that cessation rates during pregnancy and at 3 and 6 months post-partum will be significantly greater for smokers in a mood management versus the health education counseling conditions. The mood management condition is an adaptation of a therapy that has been used to treat chronic depression. The major goal of the second project, "Pharmacogenetics, Emotional Reactivity and Smoking", is to assess the effects of bupropion, nortriptyline and placebo on changes in emotional reactivity during cessation, as measured by the human startle response, EEG asymmetry and dense electrode recording of Event Response Potentials (ERP), and to determine if these effects are moderated by genotypes involved in dopaminergic, noradrenergic and serotonergic activity. He has also been awarded a competitive supplement to this grant, to evaluate the pharmacogenetic response to emotional stimuli during cessation, using fMRI.

Most pertinent to this application is the experience of the research team conducting smoking cessation clinical trials using pharmacologic agents. Our research team has considerable experience in this area. For example, we recently completed 3 multi-site FDA regulated trials evaluating the effectiveness of rimonabant, a cannabinoid antagonist, for smoking cessation, relapse prevention and reduction in selected cardiovascular risk factors (e.g. weight, lipids). Our site enrolled over 300 participants in these trials. Dr. Cinciripini is the principal investigator on one of the three trials and is responsible for conducting the data analysis and writing scientific publications of that trial and pooled results of all the trials with the drug. His work on the combined analysis of US and European rimonabant programs has been presented at an international conference [107]. He is also the lead author on one of four manuscripts being prepared on the results of these trials. The first of these manuscripts have been submitted to a leading medical journal. This manuscript deals with the results of the first US trial and showed that the drug was safe and effective. In total, approximately 789 smokers were exposed to 10 weeks of rimonabant therapy or placebo (n=790). Rimonabant 20mg resulted in a significantly higher smoking abstinence rate compared with placebo and was also associated with significantly less post-cessation weight gain. Dr. Cinciripini is has also been the site PI on a recently completed multi-site trial evaluating the effectiveness of dianicline, a partial nicotine cholinergic agonist, similar to varenicline.

We have also completed several other *investigator-initiated* smoking cessation studies using other drugs and different types of behavioral counseling. For example we evaluated the effects of venlafaxine, a norepinephrine and serotonin reuptake inhibitor, on smoking cessation. In this study, 147 smokers were randomly assigned to receive either venlafaxine or placebo in conjunction with behavioral counseling (9 weekly

sessions) and transdermal nicotine replacement therapy (22mg/day). Medication began 2 weeks prior quitting and continued for 18 weeks post quit, titrating the daily dose from 150-225mg. in response to symptoms of negative affect and relapse. The results showed no main effect of treatment on abstinence. Post-hoc analysis revealed that both at the end of treatment and at the one year follow up smokers consuming under a pack of cigarettes a day benefited from the addition of venlafaxine to the treatment regimen. Venlafaxine also reduced negative affect for all smokers for up to 6 weeks post cessation. The findings suggest that venlafaxine could have some role to play in the treatment of lighter smokers, in addition to the expected benefits of nicotine replacement therapy and behavioral counseling [23]. Other published work in this area includes studies using the anxiolytic, buspirone, which was found to have a beneficial effect on smoking cessation among smokers with high baseline anxiety[108] as well as studies combining nicotine replacement and behavioral counseling [109]. Other published work in this area includes using the anxiolytic buspirone[108] as well as studies combining nicotine replacement and behavioral counseling [109] and scheduled smoking[110].

The PI has also developed and tested a scheduled smoking as a technique for reducing cigarette consumption in a systematic way prior to cessation. This study compared the efficacy of 2 traditional methods of smoking cessation, gradual reduction and "cold turkey," with a new approach involving variation in the intercigarette interval. One hundred twenty-eight participants quit smoking on a target date, after a 3-week period of (a) scheduled reduced smoking (progressive increase in the intercigarette interval), (b) nonscheduled reduced smoking (gradual reduction, no specific change in the intercigarette interval), (c) scheduled non-reduced smoking (fixed intercigarette interval, no reductions in frequency), or (c) nonscheduled non-reduced smoking (no change in intercigarette interval or smoking frequency). Participants also received cognitive-behavioral relapse prevention training. Abstinence at 1 year averaged 44%, 18%, 32%, and 22% for the 4 groups, respectively. Overall, the scheduled reduced group performed the best and the nonscheduled reduced group performed the worst. Both scheduled groups performed better than nonscheduled ones. Scheduled reduced smoking was associated with reduced tension, fatigue, urges to smoke, withdrawal symptoms, increased coping effort (ratio of coping behavior to urges), and self-efficacy, suggesting an improved adaptation to nonsmoking and reduced vulnerability to relapse [110].

We have also completed a study combining scheduled smoking intervention, delivered on a hand held computer, with transdermal nicotine replacement. We enrolled over 800 participants in this study over a 3 year period. The results and analysis of this study are in preparation.

Maher Karam-Hage, M.D., Co-Investigator, was recently appointed as Associate Professor of Psychiatry at MD Anderson Cancer Center in the Behavioral Science and Psychiatry departments. He brings to this institution and in particular to this proposal his clinical medical experience and expertise in psychopharmacology research as well as his knowledge of addiction in general and nicotine in particular. He joined our institution coming from University of Michigan where he served for six years as Medical Director and Director of Medical Education at the Addiction Treatment and Research Centers. In addition to his medical and residency training, he had completed fellowships in addiction psychiatry and research in neuropsychopharmacology. He has completed a University of Michigan Medical School and General Clinical Research Center (GCRC) funded study, to determine the efficacy and safety of Bupropion-SR for alcoholic-smokers during the first six months of their abstinence from alcohol. He also participated in a trial of multi-site group therapy vs. nicotine patch vs. the two combined funded by NIDA-CTN (Community Trials Network) for smoking cessation in substance abusers. Starting June, 2003, he had collaborated with Cynthia and Ovide Pomerleau at Michigan's nicotine research program and served as project physician and participating investigator on three projects, including two NIDA-funded projects (R01 DA06529, Differentiation of phenotypes for smoking, and R01 DA14662, Effects of family smoking history in never-smokers) and a double-blind rimonabant efficacy clinical trial funded by Sanofi-Aventis, Inc. He most recently conducted a pilot study on cue induced cross reactivity between alcohol and tobacco with funds through the American Legacy Foundation. In his role as co-investigator in this trial he will collaborate with the PI on issues such as implementation, recruitment, medication prescribing and monitoring of response as well as side effects. He will later take a lead role in interpreting the data collected and he will join in writing manuscripts.

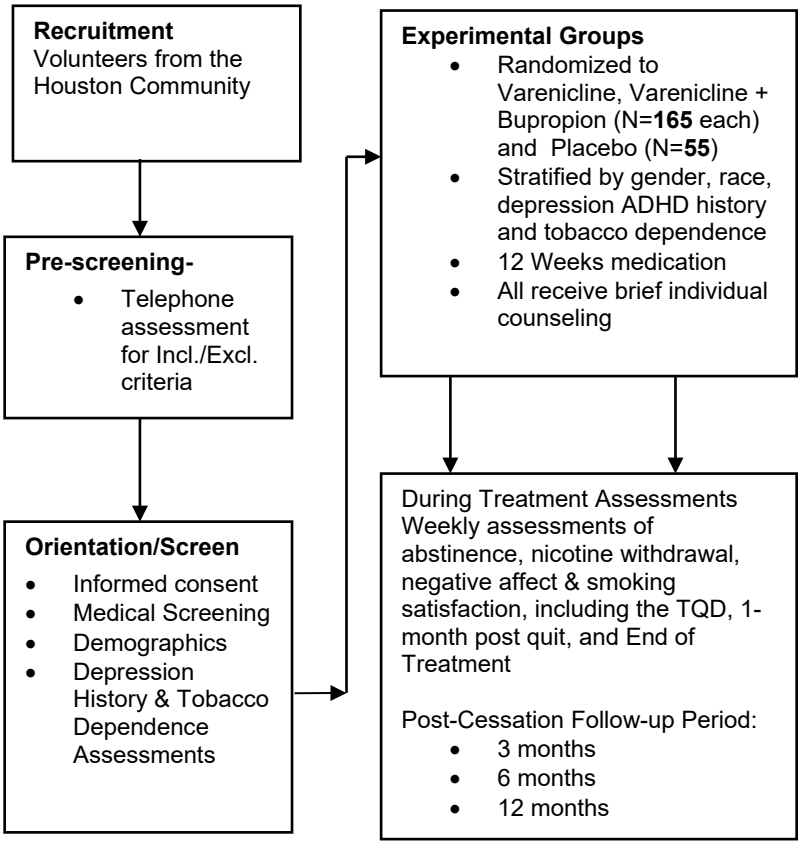
In summary, the PI and the research team he leads, has ample experience conducting smoking cessation research to adequately carry out the objectives of this proposal.

D. Research Design and Method

Design Summary

The main objectives of this study are to assess the efficacy of varenicline plus bupropion (VB) vs. varenicline (V) alone on smoking cessation and V vs. VB vs. placebo (P) on symptoms of nicotine withdrawal, negative affect and smoking reinforcement. The present study will use a double blind 3 group repeated measures design, with Group (VB/V/P) serving as the between subjects factor, and Time (Quit date, Post Cessation follow-ups) as the repeated measures. Following successful telephone and in-person screening sessions, approximately 385 eligible smokers will be randomized to receive 12 weeks of either V, VB or P, in a 3:1 ratio of active treatments (N=165 per V and VB) to placebo (n=55). Using an adaptive randomization approach (minimization)[111], the sample will be balanced with the groups, for race, gender, previous history of major depression, history of ADHD, level of tobacco dependence (smokes first cigarette within 30 minutes of waking) and history of use of bupropion for smoking cessation. Participants will receive brief in-person smoking cessation counseling at each of the 12 weekly visits at which time assessments of withdrawal, negative affect and smoking reinforcement will also be obtained. The target quit date will be set one week after beginning medication, consistent with the labeling of both medications. Both groups will attempt to quit smoking for the duration of the study if not successful on the quit date. Participants will return for follow up assessments at 3, 6, and 12 months post quit date. A study time line is provided in Table 5.

Figure 3 Design Summary and Study Flow



Subject Recruitment

Participants who want to quit smoking will be recruited from Houston metropolitan area using newspaper, radio and TV public service announcements, feature articles in the MD Anderson newsletter, and posted flyers. They will be offered modest monetary compensation for their time. We expect to have no difficulty recruiting the needed number of smokers. The MDACC Tobacco Treatment Program (TTP) is directed by the PI and has a high degree of community visibility. We have had considerable success recruiting participants for our clinical trials research.

Population Description. The population of the Houston community from which the sample will be drawn (includes Harris county) is estimated at 3,596,086 people. The ethnic distribution has been reported as 59% Caucasian (42% of which are not of Hispanic origin);19% African-American; 5% Asian; and .4% Native American, with 33% Hispanic or Latino (of any race) [112]. We expect to recruit minority smokers in proportion to the population demographics, and smoking prevalence.

Pre-Screening Telephone Assessment. All smokers will be prescreened by telephone for basic eligibility requirements (see **Table 4**). An initial description of the study design will be provided and data will be obtained on age, smoking history, other tobacco use, medical history, medication use, and pregnancy/lactation status. All subjects who remain eligible after pre-screening will be scheduled for subsequent orientation visit where the study requirements will be explained in more detail and the informed consent reviewed.

Table 4 Inclusion Exclusion Criteria

Inclusion Criteria:

- Age: 25-70 years old
- Smoking 5 or more cigarettes per day, on average, within the 2 months preceding the screening visit and expired CO ≥ 6ppm.
- Able to follow verbal and written instructions in English and complete all aspects of the study
- Provide informed consent and agree to all assessments and study procedures
- Have an address and home telephone number where they may be reached
- Be the only participant in their household

Exclusion Criteria:

- Within the month immediately preceding the screening visit, use of any form of tobacco products other than cigarettes on 3 or more days within a week if the individual refuses to refrain from such tobacco use during the course of the study.
- Within the month immediately preceding the screening visit, use of marijuana in any form on 3 or more days within a week
- Within the two weeks immediately preceding the screening visit, involvement on more than 3 days in any formal smoking cessation activities
- Treatment on a continuous basis within 2 weeks before the screening visit: any contraindicated medication for Varenicline or Bupropion. Classes of contraindicated medications include, but are not limited to, antiasthmatics, antipsychotics, some antidepressants, antihypertensives, antiarrhythmics, antineoplastics, some antiseizures, and MAO inhibitors (See Appendix U for specific list of excluded and precautionary medications).
- Uncontrolled hypertension (average reading of systolic blood pressure greater than 150 or diastolic blood pressure greater than 95) or other major contraindications for Bupropion or Varenicline (See section on Screening).
- Severe renal impairment (CR Clearance <30 ml/min/1.73 m^2).
- Laboratory evaluations outside normal limits and of potential clinical significance in the opinion of the investigator
- Meet current criteria for psychiatric disorders or substance abuse as assessed by the MINI plus (major depressive episode) and the MINI for items B, D, I, J (Alcohol Addendum-past 6 months only), K, L, M and N including a past manic or hypomanic episode as well as a lifetime psychotic disorder.
- Individuals rated as moderate (6 – 9) to high (10 or greater) on suicidality as assessed by Module C of the MINI.
- Psychiatric hospitalization within 1 year of screening date.
- A positive urine pregnancy test during the screening period. Women who are two years post menopausal, or who have had a tubal ligation or a partial or full hysterectomy will not be subject to a urine pregnancy test.
- Pregnant, breast-feeding or of childbearing potential and is not protected by a medically acceptable, effective method of birth control while enrolled in the study. Medically acceptable contraceptives include: (1) approved hormonal contraceptives (such as birth control pills, patches, implants or injections), (2) barrier methods (such as a condom or diaphragm) used with a spermicide, or (3) an intrauterine device (IUD). Contraceptive measures sold for emergency use after unprotected sex are not acceptable methods for routine use.
- Use of Varenicline or Bupropion within two weeks before the screening visit.
- History of hypersensitivity or allergic reaction to Varenicline, tricyclic antidepressant, Bupropion (Wellbutrin, Zyban) or similar chemical classes or any component of these formulations.
- Current or previous history of a seizure disorder.
- Current or previous history of anorexia.
- Subject considered by the investigator as unsuitable candidate for receipt of an investigational drug, or unstable to be followed up throughout the entire duration of the study.

Orientation Visit and Informed Consent. Ideally, the orientation visit will occur within 14 days of the telephone screen but it may occur anytime between the phone assessment and the Baseline Screening visit. A two-stage consent procedure will be utilized. During the first stage, the Primary Investigator or a trained senior level (e.g., Masters/PH.D.) staff member will present a thorough informational session to potential participants. During this session, the study purpose, other study requirements, side effects and contraindications of the medications will be reviewed. The information presented will be developed in collaboration with the Medical Doctor Co-chair of the study (Dr. Maher Karam-Hage) and will be based on current studies using these drugs, as well as manufacturer and PDR information. Participants will be given the opportunity to ask questions about the informed consent document or any aspect of the study. Any medical questions that arise during the process, if not addressed in the documentation or discussion provided, will be referred to the medical staff and the information will be provided to the potential participant prior to consenting. If a subject expresses continued interest in participating in the study, they will be asked to schedule their baseline screening appointment at this time.

Screening Overview. At start of screening visit, the informed consent document will be reviewed with the subject and they will be asked to sign the informed consent document. Additionally, the subject will be assessed for interest in providing a buccal sample according to procedures described in our IRB-approved genetic banking protocol (Protocol # Lab 09-0099). If the subject agrees to provide one by signing the informed consent approved for that protocol the sample will be collected at Visit 1. As shown in Table 5 subjects will be screened to assess medical and psychiatric suitability for the study. Medical screening for potential contraindications for medication use will be conducted by our study physician and/or in conjunction with other qualified members of the medical

staff (e.g., RN, PA, or APN). These conditions include the presence of hypersensitivity to either medication; use of MAO inhibitors or discontinuation within the past 2 weeks, recent myocardial infarction, uncontrolled hypertension, tachycardia, hypotension or risk of orthostatic hypotension, clinically significant renal or hepatic disease, use of other psychotropic medications including some antidepressants, or drugs that inhibit the P450 enzyme system and previous history of a seizure disorder. Based on the MINI or MINI Plus (see below) and/or the presence or absence of psychotropic medication, or evidence in the patients health history and/or interview, we will exclude serious and unstable psychiatric conditions, including schizophrenia, bipolar disorder, current major depression, patients at risk for suicide, as well as other disorders such as panic disorder, PTSD, alcohol or substance dependence. We will also exclude those with anorexia as this is a contraindication for bupropion. Many of these disorders are treated with medications that are contraindicated for the use of bupropion and given the need to accurately describe the AE profile in the combination treatment, concurrent medications are kept to a minimum, exclusive of those used to manage chronic systemic medical conditions such as hypertension, hyperlipidemia etc. Standard blood chemistries including liver and renal function tests will be ordered on the initial screening session and reviewed prior to the subsequent visit for acceptability.

At the Baseline Screening (Visit 0), which will occur within 30 days of the telephone screen, study participants will be asked to provide medical/surgical history, smoking history and to complete other assessments as shown in Table 5. They will also be asked to provide a blood sample so that liver and kidney function can be assessed. Participants unable to complete the Baseline screening visit within30 days of the initial telephone assessment will be allowed to undergo a second telephone assessment and given an additional 30 days to complete the Baseline screening visit. If they do not complete the Baseline by this time point, they will no longer be considered eligible for study participation and must undergo a 90-day waiting period to re-enroll. Participants will not be randomized into the study at baseline screening given that they must complete the medical screening and the results of the lab work must be received before final eligibility can be determined. Participants who remain eligible after the baseline screening visit will be scheduled to return for the second in-clinic (medical) screening visit (Visit 1) within 7 – 21 days. If initial lab values are abnormal, blood work may be repeated before V1. Study physicians will use the results of the second blood draw to determine the accuracy of the initial values and to make a more informed decision regarding study eligibility. If a participant is eligible for another one of our research studies but is not enrolled in that study, the MINI, blood chemistries, and blood pressure collected for that study will be considered valid for 30-60 days and may be used in consideration of determining initial eligibility on this study.

The Medical Screening (Visit 1) will occur within 21 days of the Baseline Screening visit to determine final eligibility. During Visit 1, the Research Nurse (RN) will review with the participant information collected at the baseline screening visit (e.g., medical/surgical history, psychological history, etc), results of blood work, vital signs and concomitant medications to ensure the accuracy of the information reported and to make sure there is nothing new to add. The RN will also conduct a Review of Systems (ROS), head, ears, lungs, heart, etc., to determine the participants' current level of health. Once the medical history review and ROS are complete, the RN will page/call the study physician or another qualified member of the medical staff (e.g., PA or APN) for a consultation regarding the participants' medical history, concomitant medications, and the results of the ROS. The study physician (or another qualified member of the medical staff as spelled out above) will make a determination as to whether a full physical examination is needed. The following criteria will be used to make that determination: (1) History of a neurological disorder (e.g., migraines, head trauma, etc), (2) History of chest pain or other major cardiovascular event, (3) Presence of a chronic illness (e.g., diabetes, HIV, hypertension), or (4) Any condition or new information that is of concern or that requires further clarification, based upon the professional opinion of the medical staff. In these cases, a full physical examination will be conducted. Participants will also be given the opportunity to ask questions of the medical staff during this visit. If it is determined that an individual is eligible for study participation, the medical staff who conducted V1 will sign a medication prescription that will be filled by the pharmacy at M. D. Anderson Cancer Center as per protocol. The medical staff (RN, PA, APN, or study physician) will also provide written documentation (dictated to medical chart) that the PA, APN, or study physician cleared the potential subject to participate on the protocol and the subject will be randomized into treatment. If a person is deemed ineligible, s/he will be given

a letter stating the concern(s) and referred to his/her personal physician for follow-up. Medical personnel may also delay clearance until any screening issues, which are not exclusionary, have been addressed by the potential subject's personal physician.

On occasion, V1 randomization may be delayed beyond 21 days from the Baseline visit due to medical findings that may be significant but not exclusionary, participant-related events (e.g., travel, work schedules), or study related factors (e.g., full clinic schedule). In these cases, participants' pregnancy status, blood pressure, concomitant medications, mental health history, medical/surgical history, and smoking history will be reviewed before completing the randomization. If there are no significant changes, participants will be allowed to continue to the V1 randomization visit as described above. If there are significant changes, the baseline screening process will be conducted again as deemed appropriate by the medical team.

Re-Visit Schedule and the Visit Window

Visit 2 will occur nine days after start of medication. Following Visit 2, in-clinic treatment visits will be conducted weekly for Visits 3 - 13. Every effort will be made to have the subjects return to the clinic on the same day of the week for study visits, however, a +/- 3 day window will be permissible for all return treatment visits except Visit 2 which will only have a +3 day window. If the end of the +/- 3 day visit window falls on a weekend, holiday, or other day on which the clinic is officially closed, subjects will be allowed to complete the visit during the next business day except for V2 which will not be scheduled beyond +3 days. Follow-up visits will have expanded visit windows such that the 3-mo Follow-Up Visit has a window of +2 weeks or -13 days and the 6-mo Follow-Up visit has a window of +/- 1 month. The 12-mo Follow-Up Visit has a window of -1 month with an ending period of whenever final abstinence data is collected or the trial ends, whichever is sooner. For those that miss a follow up visit, staff will call them to invite them to come in as originally planned so that at minimum abstinence data such as the time-line follow back assessment, CO and saliva cotinine can be collected. For those in follow up that report current abstinence and that they will not or cannot come in to complete the visit, we will ask for their consent to send them an abstinence questionnaire and cotinine saliva collection kit which they can conduct themselves and mail back to us. In exchange for their completed abstinence packet (See Appendix VV and WW), we will mail them a gift-card for $20.

Start of Medication and Medication Tracking

Subjects will begin self-administration of medication 1 to 10 days after Visit 1 and will track their medication dosing and cigarette consumption throughout the study using smoking diaries that will be provided to them by the study counselor (See Appendix Y). Ideally, subjects will take medications for a full seven days before the target quit date (TQD), which occurs on day eight of treatment.

Early Termination of Study Drug

If early termination of study drug is necessary and occurs before the end of the 12-week treatment period (Visit 13), an early termination visit (ET 13) will be conducted in order to collect the end-of-treatment lab work and other evaluations, regardless of whether the subject continues in the study. If a subject prematurely and permanently discontinues study drug, they will be encouraged to continue to be followed in accordance with the study protocol through the end of the study.

Disposition of Study Drug

Occasionally, research subjects may fail to take a scheduled dose of medication. In such cases, subjects will be instructed to mark the missed dose(s) in their smoking diaries and return the missed dose(s) of medication to the study team. Returned medications will be logged, stored in a locked file cabinet maintained by the study team, and will be destroyed per institutional guidelines. Unused medications (i.e., those that were never dispensed by Investigational Pharmacy Services) will be maintained by the pharmacy team and will be destroyed per institutional guidelines.

Subject Withdrawal

A withdrawal occurs when an enrolled subject ceases participation in the study prior to completion of the protocol, regardless of the circumstances. Subjects may withdraw from the trial at any time at their own request, or they may be withdrawn at any time at the discretion of the investigator for safety reasons. All attempts will be made to collect medications, and to conduct the final laboratory and other evaluations required by the protocol at the time of withdrawal.

Assessments and Questionnaires

MINI International Neuropsychiatric Interview and the MINI Plus. Current psychiatric disorders will be ruled out using version 5.0 of the MINI [113]. The MINI screens for several DSM-IV Axis 1 diagnosis including depression, anxiety, bipolar and eating disorders, as well as substance and alcohol abuse. The MINI also provides a graded assessment of suicidality (low-high risk). The MINI is shorter and takes less time to administer than the Structured Clinical Interview for DSM-IV disorders (SCID) [114] but has been validated against that instrument [22,113]. Module A from the MINI Plus will be used to assess current as well as past history of depressive episodes even though evidence of past history will not be exclusionary, it will be taken into consideration should participant report any psychiatric adverse events while in treatment.

Conners Adult ADHD Rating Scale (CAARS). We will also rate current ADHD symptoms using the Conners' Adult ADHD Rating Scale (CAARS) [115]. This is a validated self-report instrument that has been used in studies of adult ADHD [116]. Each of its 18 items corresponds to one of the 18 DSM-IV symptoms for ADHD and is rated on a 4-point scale. As shown in Table 5, the scale will be administered at several points during the course of treatment to track possible changes due to treatment. Several of the cognitive symptoms related to concentration and restlessness etc. are also evaluated as part of the nicotine withdrawal assessment.

Table 5. Study Time Line and Procedures

STUDY TIME FRAME	SCREENING			TREATMENT													FOLLOW-UP* (3 6 &12 months Post-Quit)		
Visit # (See 'note' below)		-1	0	1[a]	2	3	4	5	6	7	8	9	10	11	12	13[b,c]	14[d]	15[e]	16[f]
Study Day	-28	-14	1[g]	8	17	24	31	38	45	52	59	66	73	80	87	94	107	197	382
Days from Quit	-42	-28	-14	-7	1	8	15	22	29	36	43	50	57	64	71	78	91	181	366
Days on Medication					9	16	23	30	37	44	51	58	65	72	79	86			
ASSESSMENTS																			
Phone Screen (Basic Eligibility)	X																		
Orientation		X																	
Medical & Other Screening																			
Informed consent signed		X																	
Demographics			X																
FTND			X																
ERQ			X																
CERQ			X																
Smoking History			X																
Medical History			X																
Blood Chemistry			X				X									X			
Motivation Questions			X																
Pregnancy Test			X																
Height			X																
Weight			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Blood Pressure/Heart Rt			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
ROS/Physical Exam				X															
MINI & MINI Plus			X																
Hx of ADHD (Kollins)			X																
Abstinence Questionnaire*			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

Conner's (CAARS) Adult ADHD				X	X		X		X							X			
WSWS				X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
PANAS				X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
CES-D				X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
QSU				X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
mCEQ**				X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
WiSDM			X																
Sleep Problems Scale			X					X				X				X			
Cognitive Assessment Tasks***				X	X		X		X							X			
EEG Assessments				X	X				X										
Smoking diary				X	X	X	X	X	X	X	X	X	X	X	X	X			
Buccal Sample				X															
Saliva Cotinine				X	X											X	X	X	X
Expired CO			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Pill Count					X	X	X	X	X	X	X	X	X	X	X	X			
Adverse Events*				X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Symptoms Checklist				X	X	X	X	X	X	X	X	X	X	X	X	X	X		
Concomitant Meds*		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
TREATMENT																			
Counseling				X	X	X	X	X	X	X	X	X	X	X	X	X			
Dispense Medication****				X			X				X								

a-Randomization visit; start medication the next day; bc- End of Treatment or ET 13 visit (Visit 13; Week 13); d-3month post quit follow-up (Visit 14; Week 15); e-6 month post quit follow-up (Visit 15; Week 28); f-12 month post quit follow-up (Visit 16; Week 54); g-within 30 days of initial phone assessment but eligible for rescreen and an additional 30 days if needed; * Brief telephone contacts at 1-day pre-quit, 3 days post-quit and at Weeks 14, 20, 34, 42, and 48 to provide counseling support (1-day pre-quit and 3-day post-quit phone calls only), and to assess adverse events (Week 14 and Week 20 phone calls only), cigarette and other tobacco use, as well as use of medications for smoking cessation**. Note:** Subjects will be given a +/- 3-day window in which to complete a visit before the visit is considered missed (see protocol for additional details). **Only administered to participants that indicate non-abstinence since last visit***If missed at designated visit (due to patient no show, error, computer malfunction, etc.) task will be conducted at subsequent visit unless task already designated for that visit****Medication dispensing will occur in bulk quantities at V1and then again at V4 and V8. If pt cannot come at V4 or V8, meds will be dispensed when possible.

History Of ADHD. Smokers will be evaluated for a history of ADHD using the rating scale from the National Longitudinal Study of Adolescent Health, a large-scale epidemiological study of ADHD and smoking, and described by Kollins and colleagues [48]. The scale retrospectively assesses childhood ADHD symptoms in adults. The 9 DSM-IV symptoms of Inattention (IN) and 8 DSM-IV symptoms of Hyperactivity/Impulsivity (HI) are rated for past frequency of occurrence. Symptoms rated as having occurred "often" or "very often" are considered to have been present. Six or more symptoms from either the IN or HI categories are needed to qualify for positive history of ADHD.

The Demographic, Health and Smoking Health Questionnaires. These instruments expand on the data obtained during the pre-screening, providing more detailed information on demographics, health/medication history, alcohol, caffeine, and other drug use, for use in the medical screening. Information on smoking history (e.g., year's smoked, previous quit attempts, relapse, current smoking rate, and other nicotine/tobacco use) is also obtained. These questionnaires have been used in our previous and current cessation studies to provide descriptive data for the study population (e.g., [109,110]).

The Fagerstrom Test for Nicotine Dependence (FTND). The FTND is a 6 item questionnaire that measures nicotine dependence by assessing various components of smoking behavior such as daily intake, difficulty in refraining from smoking, and time to first cigarette [117,118]. In some studies, the scale has been found to correlate with cotinine level [119] and to predict smoking treatment outcome [120]. It was modified from the most commonly used nicotine dependence measure, the Fagerstrom Tolerance Questionnaire [121]. Time to first cigarette (within 30 minutes) has been noted to be the item accounting for the majority of the variance in this scale and the one most highly correlated with multidimensional scales of nicotine dependence [122].

The Wisconsin Inventory of Smoking Dependence Motives. Questionnaire (WISDM) [123] yields an overall nicotine dependence score as well as subscale scores for other critical dimensions of tobacco dependence (cognitive enhancement, negative reinforcement, positive reinforcement, automaticity, affiliative attachment, loss of control, behavioral choice/amelioration, craving, cue exposure/associative processes,

social/environmental goals, taste/sensory processes, weight control, and tolerance). The scale was developed using samples of daily smokers and validated on both clinical and non-clinical samples. As such it is highly sensitive to predictors of daily smoking level, “heaviness of smoking” expired CO, as well as abstinence following treatment.

As shown in **Table 5**, participants will be asked to complete additional assessments throughout the course of the study. Brief descriptions of these assessments are provided below.

The Wisconsin Smoking Withdrawal Scale (WSWS). The WSWS [124] will be used to assess withdrawal symptoms. We will use the Anger, Anxiety, Concentration, and Sadness subscales of WSWS, and the Craving subscale to ascertain the effects of quitting on mood and urges to smoke, respectively. The WSWS has scale coefficient alphas between 0.75 and 0.93 and the mood and craving scales demonstrate increases as a function of nicotine abstinence as well as prediction of treatment outcome. Items from the Minnesota Withdrawal [125] are included in the scale for comparability to earlier studies using that measure.

The Positive and Negative Affect Scale (PANAS). The PANAS [18] is comprised of two 10-item mood scales: Positive Affect (PA) and Negative Affect (NA). Participants rate different feelings and emotions on a scale of 1-5. Various time instructions (e.g., today, past few days, past week, general, etc.) have been used with acceptably high alpha reliability ranging from .86 to .90 for PA and .84 to .87 for NA. Post-cessation PANAS negative affect is a robust predictor of relapse[23,68]

The Center for Epidemiologic Studies Depression Scale (CES-D). The CES-D is a 20-item self-report measure developed to assess depressive symptoms in community (nonclinical) populations [126] and in recent studies of smoking cessation [127]. This scale consists of four factors: depressed affect, enervation, lack of positive affect and interpersonal problems.

Questionnaire on Smoking Urges -- Brief Version (QSU). The QSU Brief Form is a 10 item validated questionnaire measuring desire and intention to smoke; and anticipation of relief from negative affect and desire to smoke. The QSU has been found to be predictive of craving in laboratory studies [128] and lower scores on each of the subscales have been noted for smokers treated with varenicline or bupropion in the original clinical trials of varenicline [4,6].

Modified Cigarette Evaluation Questionnaire (mCEQ). The mCEQ is a 12 item self-administered instrument providing ratings of a smokers most recent smoking experience on multi-item domains of Smoking Satisfaction (satisfying, taste good, smoking, enjoyment), Psychological Reward (calm down, more awake, less irritable, help concentrate, reduce hunger), and Aversion (dizziness, nauseous), as well as a single-item assessment on Enjoyment of Respiratory Tract Sensations and on Craving Reduction, using a scale of 1 (not at all) to 7 (extremely). The original version contained 11 has been shown to be sensitive to interventions that purportedly reduce the pleasure associated with smoking (i.e., the nicotine antagonist mecamylamine and denicotinzed cigarettes [129,130]. A factor analysis of the mCEQ in which a 12th item (enjoyment) was added to the satisfaction subscale was carried out using data from the 3 pivotal varenicline trials [131] , This analysis demonstrated the validity and, in general, the reliability of the multidimensional framework of the mCEQ. Cronbach's alpha for internal consistency exceeded 0.70 for all scales except Aversion domain. Test–retest reliability exceeded 0.70 on the three multi-item domains and two single items. Moreover, varenicline (and bupropion) has been shown to reduce smoking satisfaction and reward, relative to placebo, although the effect sizes were somewhat larger for varenicline.[4,6]

Motivation Scale. This is a 10-item assessment created specifically for smoking cessation research to measure a participant’s motivation to quit smoking.

CERQ-short. The CERQ-short is an 18 item validated questionnaire that assesses an array of cognitive emotional regulation strategies and consists of 9 subscales: self-blame, acceptance, rumination, positive refocus, refocus on planning, positive reappraisal, putting into perspective, catastrophizing, and other-blame. These regulation strategies have been related to a variety of affective outcomes and may be related to cessation rates in this study.

ERQ. The ERQ is a 10-item validated questionnaire that assesses individual differences in two emotional regulation strategies: cognitive reappraisal and emotional suppression. These strategies have been related to negative affect, which may be related to cessation rates in this study.

Sleep Problems Scale. This 4-item scale, designed to assess symptoms of sleep disturbance as a function of treatment and nicotine withdrawal, will be administered at baseline and again at 4 weeks, 8 weeks, and 12 weeks after treatment begins.

Cognitive Assessments. Cognitive performance will be assessed using the Attention Network Test (ANT), and the Rapid Visual Information Processing Task (RVIP-CED). The tasks are programmed using E-Prime, Version 1.1 143and take about 15 minutes to complete (in total).

The Attention Network Test (ANT) has been implemented following the procedure used by Fan et al. {Fan, 2005 8027 /id}. The task lasts for approximately 20 minutes and it is divided into four blocks with resting pauses in between blocks. The first block is a practice block. The task has been implemented using Eprime software. In each trial, a fixation cross appears in the center of the screen all the time. Depending on the cue condition, a cue (none, center, or spatial cue) appears for 200 ms. After a variable duration (300– 11800 ms), the target (the center arrow) and flankers of left and right two arrows (congruent or incongruent flankers) are presented until the participant responds with a button press, but for no longer than 2000 ms. After the participant makes a response, the target and flankers disappear immediately and a post-target fixation period lasts for a variable duration (from the onset of the target and the start time of the next trial is between 3000 and 15000 ms).

The Rapid Visual Information Processing Task (RVIP-CED). proposed in this trial consists of numeric digits (1-9) which will be presented every 600 ms (100/min) at the center of the computer monitor interspersed with slide distracters (pleasant, unpleasant, neutral and cigarette pictures that last 600 ms each. The participant will be instructed to push a response button if either three consecutive even or odd digits are shown. The task will last approximately 15 minutes.

Slide Distracter Stimuli. The distracter slides are presented immediately prior to some of the RVIP digits in order to evaluate their impact on smokers' attention to the RVIP task {Gilbert, 2005 6717 /id}. The distracter slides will be composed of four types, including smoking, pleasant, unpleasant, and neutral content. The pleasant, unpleasant, and neutral slides will be drawn from the International Affective Picture System (IAPS {Center for the Study of Emotion and Attention, 1999 4947 /id}). Additional cognitive assessments are provided by the CAARS as indicated in Table 5.

Laboratory Assessment of Cognitive Response. Electroencephalography (EEGs) assessments will supplement the ANT, RVIP-CED and CAARS as an additional measure of cognition. The protocol for the individual EEG assessments will be similar for all sessions. Each session will begin at approximately the same time of day for each participant**.** Participants who are selected to participate in this part of the protocol will be asked to limit their intake of coffee (or equivalent) to no more than 1 cup prior to 8:00 am on the day of each laboratory session. Smoking for all groups will be unrestricted prior to the first (baseline) EEG assessment session (V1). The first session will be used to assess normal EEG responses to affective stimuli prior to any nicotine deprivation or pharmacological treatment. Subsequent sessions will be used to assess the effects of treatment modality (Varenicline, Varenicline plus Bupropion, placebo) on EEG responses to affective stimuli. The target days for these laboratory assessments are one week pre-quit (V1), one-day post-quit (V2), and one-month post-quit (V6). At the beginning of each EEG session, participants will be asked to complete the session questionnaires. After completing the questionnaires, sensors will be placed on the scalp to measure electroencephalography (EEG). Participants will be asked to rest quietly, while seated in a comfortable recliner, so that baseline data can be measured – with eyes open and with eyes closed. The subject will be told that a series of slides will be presented and that each slide should be viewed the entire time it is on the screen. Participants will sit approximately six feet from the viewing screen and will see a variety of pictures. Each slide will be presented for approximately 6s, followed by a randomly determined interslide interval, 1.5 to 20 seconds in duration. The images will be selected from the International Affective Picture System (IAPS) (Center for the Study of Emotion and Attention, 1995) and are the same as used in our preliminary studies. These slides have been standardized for valence and arousal (e.g.,). The smoking cue slides (lit cigarette, ash tray, people smoking, etc.) were developed in our laboratory for our preliminary study and will also be used here. The slides will be equally divided among the valence categories and the smoking cues. Different slide sets will used in each session with the order of set presentation counterbalanced across subjects. Within each set, each subject will see pictures from each category equally often in each position. Counterbalancing for slide order within a set will be arranged. After the final block, participants will view an additional set of slides and will be

asked to rate the valence of the pictures. EEG physiological recordings from this task will be scored offline for early and late components of the ERP. These components are sensitive to cognitive and emotional processing of visual stimuli. EEG assessments will not be done until funding becomes available and will only be conducted in up to 120 subjects.

Upon completion of each questionnaire set, participants will be asked to sign an acknowledgement form in which they will indicate and acknowledge the assessments they just completed. (See Appendix HH).

EEG Data acquisition and reduction. During the execution of the task, the electroencephalogram (EEG) will be recorded using a 129-channel Geodesic Sensor Net and amplified with an AC coupled high input impedance (200 MΩ) amplifier (Geodesic EEG System 250; Electrical Geodesics Inc., Eugene, OR) vertex referenced to Cz. Sampling rate will be 250 Hz and data will be filtered online using a 0.1 Hz high pass filter. After data collection, a 100 Hz low pass filter will be applied to the recording and an artifact detection procedure will be used following the method proposed by Junghöfer and colleagues [132]. This procedure detects artifacts by performing two passes on the data. The first pass uses the original reference (i.e., Cz), to avoid contamination of all channels by the artifacts when transforming EEG data to the average reference. The second pass uses the re-referenced data. At the end of the first pass, sensors consistently contaminated by artifacts will be interpolated using the information contained in the uncontaminated sensors. After this first pass, the average reference (necessary for accurate topographic mapping and topographic waveform plots) will be calculated and the second pass will be performed to identify contaminated sensors within specific trials. If, within each trial, more than 5% of the sensors are artifact contaminated, the trial will be rejected; otherwise the contaminated channels will be interpolated. After these steps individual epochs, time-locked to the stimulus onset, will be extracted from the continuous EEG. Event-Related Potentials (ERPs) will be computed individually for each participant according to the experimental condition. ERP components (e.g., P1/N1, P300, late positive potential) will be identified and average voltage within specific time windows will be computed and used in the statistical analyses.

Abstinence Questionnaire & Expired CO. The Abstinence Questionnaire is a 16-item interview administered questionnaire that will be used to assess smoking behavior and abstinence throughout the course of the study. Abstinence will be verified by an expired CO reading of < 10ppm at each in-person measurement occasion. This questionnaire has been used in our previous studies and is administered using a computer. The program utilizes subject specific events (birthdays, anniversaries etc.) and general milestones (holidays) which allows the interviewer to conduct a time-line follow back procedure to assess all smoking in between visits. All measures of abstinence as described can be assessed using this procedure.

Cotinine. Cotinine is the first metabolite of nicotine and has a half-life of about 20 hrs[134]. Saliva cotinine will be obtained at the time of randomization and at selected follow-up points shown in Table 5. Cotinine values will provide information regarding the participant's tobacco exposure within the previous 24-36 hours. Baseline cotinine values will be used in the descriptive analyses of smoker characteristics, along with other variables from the smoking history questionnaire. Cotinine values (<25ng/ml) from subsequent assessments will be used as a crosscheck on abstinence requirements during follow-ups. Cotinine assays will be performed by Salimetrics in State College Pa. This is a reliable lab affiliated with Penn State and one which we have successfully used in several previously studies. We anticipate no problems in transporting samples or obtaining valid results.

Adverse Event Monitoring & Concomitant Medication

Participants will be assessed for side effects and concomitant medications using standard FDA guidelines recommended for these two procedures. Additionally, from V1 through V14, a symptoms checklist will be administered to quickly assess for certain symptoms a participant may be currently experiencing or have experienced since the last visit that may require immediate follow-up by someone from the study medical team before the study visit is concluded (see Appendix II). Blood pressure, heart rate, and weight will be monitored weekly during treatment and at follow-up. We consider a sustained blood pressure (BP) > 150/95 the stopping criterion where this measure is concerned. That is, if a subject has an average BP> 150/95 for three consecutive study visits, the study physician will be contacted to evaluate whether the study medication should be discontinued, a change in dose ordered and/or whether the subject should be referred to his/her personal physician for follow-up. The subject would not be discontinued from the study (see Appendix BB for details). If

there are clear reasons for the elevation, such as the patient failing to take their antihypertensive medication, they may be re-challenged with the study drug, at the study physician's discretion once their BP is controlled. Additionally, if a subject has a sustained (for three consecutive study visits) average reading of systolic BP of 140 – 149 and/or a sustained diastolic BP of 90 – 94, the study physician will be contacted to evaluate whether the medication should be discontinued, a change in dose ordered and/or whether the subject should be referred to his/her personal physician for follow-up. The subject will not be taken off study drug unless the study physician or his/her personal physician recommends that we do so. Liver (LFT's) and renal function renal function (creatinine clearance) will be evaluated at baseline, after 3 weeks on the medication(s) and at the end of treatment. Blood chemistries will be repeated as needed accordingly when clinically warranted.

Adverse events will be reviewed by our medical personnel or the PI and the physician along with the PI may adjust medication doses to manage subject complaints of intolerable or untoward side effects. Medication doses may also be adjusted if subjects' reports of side effects cause concern even if there are no specific subject complaints. Dose adjustment strategies will follow good clinical practice guidelines as recommended for management of side-effects for these two medications. This may include reduction to once per day dosing on the maintenance dose(s), twice per day dosing at a lower dose(s), stopping medications altogether, or any other approach the medical team deems most appropriate to manage the side effect profile. For example, in the VB group one or both medications may be dose adjusted or medications may be stopped altogether if the side effect profile and/or subject complaints warrant it. Adverse event monitoring will continue up to 90 days after medication is completed, which corresponds to the 6-month post-quit visit (V 15). If an AE is spontaneously reported after the AE reporting period is over, the AE will be recorded. Those AEs that are probably, possibly or definitely related to treatment will be followed until resolution or end of study, whichever comes first. In the case of reports of suicidal ideation, depression or anxiety which we believe may be related to treatment, if possible, we will engage in our normal psychological assessments (see Appendix S). This may not always be possible if reported by phone. In any case, procedures for Good Clinical Practice will be followed with respect to medical management of the symptoms. A specific plan for monitoring increased depression and suicidality is presented in the appendix (see Appendix S). Details regarding the monitoring/management of treatment-emergent anxiety are also included in Appendix S. The clinical assessment tool we use is the HAD (the Hospital Depression and Anxiety Scale) and the procedures outlined in Appendix S apply to both symptoms of anxiety and depression. Moreover, because aggression and irritability are intimately associated with anxiety, these symptoms are also captured in our monitoring and management procedures. Treatment-emergent psychosis is extremely rare in smoking cessation trials. If it does occur, it will be captured by our weekly assessment of adverse events. These cases will be referred for further evaluation by a Ph.D. licensed Clinical Psychologist and/or the Addiction Psychiatrist, who is a co-investigator on this protocol. The Addiction Psychiatrist will determine the course of clinical management according to methods of good clinical practice.

The PI or physician is responsible for determining the attribution of adverse events to study medication. For this trial, AEs will be recorded according to the Recommended Adverse Event Recording Guidelines for Phase III protocols (see shaded areas of **Table 6**).

Table 6. Recommended Adverse Event Recording Guidelines

Attribution	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
Unrelated	Phase I	Phase I	Phase I Phase II	Phase I Phase II Phase III	Phase I Phase II Phase III
Unlikely	Phase I	Phase I	Phase I Phase II	Phase I Phase II Phase III	Phase I Phase II Phase III
Possible	Phase I Phase II	Phase I Phase II Phase III	Phase I Phase II Phase III	Phase I Phase II Phase III	Phase I Phase II Phase III
Probable	Phase I Phase II	Phase I Phase II Phase III	Phase I Phase II Phase III	Phase I Phase II Phase III	Phase I Phase II Phase III
Definitive	Phase I Phase II	Phase I Phase II Phase III	Phase I Phase II Phase III	Phase I Phase II Phase III	Phase I Phase II Phase III

<u>Concomitant use of varenicline & Bupropion.</u> Bupropion is hydroylated into hydroxybupropion, its active form via the enzyme CYP2B6, and bupropion is a cytochrome p450 2D6 inhibitor. Nicotine was first found to be an inducer of CYP2B6 in rat brains and a 2003 study suggests that the same affect is seen in human brains. These data are interesting in that higher levels of CYP2B6 can be thought to increase the levels of active form of bupropion that would be seen in a normal brain. This could lead to an elevated effect

of the drug and thus increased efficacy[135]. However, pharmacokinetic studies of varenicline show that it is metabolized extensively and that 92% of drug-related material is excreted unchanged in the urine. The straightforward dispositional profile of varenicline simplifies adding bupropion to it without any foreseeable complications or competitive metabolism [136]. The concomitant use of varenicline and bupropion has been tested among 46 smokers in a pre-marketing study conducted by Pfizer (see www.chantix.com). These subjects were not instructed to quit smoking and began both varenicline and bupropion SR using the same dosing schedule for the first two weeks as described in this protocol. The results showed that varenicline did not alter the pharmacokinetics of bupropion nor were there any clinically significant adverse reactions observed during the period of co-administration. The primary AE's for either drug (nausea for varenicline and insomnia for bupropion) were as expected for either drug alone. There are also post-marketing reports of an increase in depression, anxiety and suicidal thoughts while on varenicline. As discussed above a proper plan for monitoring and responding to such symptoms has been established.

Assessment Protocol and Participant Burden. All assessments will be conducted by non-counselor research staff (e.g., research assistants, nursing and medical staff, etc), who like other staff are blind to medication assignment. This procedure eliminates the likelihood of counselor bias on the outcome assessments. Self-report measures are administered on a computer-based program. To reduce the potential frequency of missing data, we follow a standardized procedure for contacting the participant with appointment reminders by telephone and letter and in the event a session is missed every attempt is made to reschedule the visit within a 3 day window. As a last resort, telephone visits will be conducted but no compensation will be earned for these phone visits. In these cases Adverse Events, Concomitant Medications, Abstinence Questionnaire, Symptoms Checklist and counseling will be assessed/administered. The entire assessment protocol should take no longer than 45 minutes. We have conducted numerous trials using these and many other psychological and cognitive assessments with little problems in participant compliance. Past experience has shown us that the longer a participant is enrolled, the greater the chance that they will fail to complete a visit. So, although we strive to ensure patients complete each visit, we expect that some will not and as such, we will not require every visit be completed in order to participate in the study.

Treatment Intervention

Counseling Component Behavioral counseling is a recommended standard of care to be used in conjunction with pharmacotherapy [137]. At visit 1, the counselor will provide the participant with a card with emergency contact information, study medication instructions and a participant manual which they will refer to throughout treatment. As shown in Table 5, individual brief-behavioral counseling sessions (10-15 minutes) will be provided to all participants once per week for 12 weeks. One support phone call will also be conducted 3 days after the target quit date. The counseling intervention is modeled after programs that have been used in several smoking cessation medication studies, including bupropion [128,138], nortriptyline [139], venlafaxine [23], rimonabant [107] and varenicline[4,6]. Our individual counseling sessions are comparable in duration and scope to that used in the pivotal trial contrasting varenicline and bupropion[4,6], although in those trials counseling was more frequent than that used in the original trial of bupropion (7 weekly individual counseling sessions, 1 call, 10-15 minutes each) [138], since a longer duration of medication was used (12 weeks). This is meant as an example and will be modified accordingly to reflect appropriate time line, number of visits phone support and drug information. In addition to the 3-day post quit support call, there will also be one pre-quit day phone call and five follow-up assessment phone calls during the course of the study. If unable to reach participant during first call attempt, the visit will be considered missed and no further attempts will be made for the 1 day pre-quit and the 3 days post-quit calls as the visit window is only 1 day. For the remaining five follow-up assessment phone calls, the first two phone calls will observe an additional 1-2 call attempts within the 3-day window before the visit is considered missed. The last three phone calls will observe an additional 2-3 call attempts within the +1 month window before the visit is considered missed. Although we strive to ensure patients complete each visit, we expect that some will not and as such, we will not require every phone visit be completed in order to participate in the study.

Varenicline/Varenicline plus Bupropion/Placebo Smokers will receive a 12-week regimen of V, VB or P beginning the day after they complete the second screening visit (Visit 1). During the first week of medication the dose will be titrated, as recommended in the dosing guidelines for both drugs. Smokers in both groups V and VB will take 0.5mg varenicline once per day (am) for the first 3 days and 0.5mg bid (am and pm) for the

next 4 days. Smokers in the VB group will also take 150mg bupropion (SR) once per day (am) for the first 3 days followed by150mg bid for the next 4 days. Smokers in the V group will also take a matching bupropion placebo on the same schedule as those in the VB group. For safety reasons, if a participant misses any dose of medication during a ramp-up phase, the PI and/or Study Physician may re-evaluate whether the start or change of medications and/or the Quit Date should be delayed. From week 2-12, smokers in the V group will take 1mg of varenicline and one bupropion matching placebo twice per day. Smokers in the VB group will take 1mg of varenicline and one active 150 mg bupropion tablet twice per day. Smokers in the P group will take a matching varenicline and bupropion placebos on the same schedule as the V group takes varenicline and the B group takes bupropion, respectively. Bupropion and matching placebo will be made by over-coating the active medication and matching placebo so that they appear identical. Varenicline and matching placebo will appear identical in size, color, and shape to maintain blinding. Varenicline and matching placebos will be provided by Pfizer pharmaceuticals and will be encapsulated by a compounding pharmacy. We currently use a similar procedure in our on-going R01 using pharmacogenetic trial of bupropion, varenicline and placebo, and have an established relationship with a pharmaceutical compounding company to provide this service. Bupropion (SR) is now in generic form and no matching placebo is available directly from the generic manufacturers. Varenicline will be purchased and prepared by the compounding pharmacy and delivered to Investigational Pharmacy Services, which will manage the inventory for dispensing by the Outpatient Pharmacy. The MD Anderson pharmacy will implement the randomization schedule as developed by the study data manager. Our pharmacists have considerable experience preparing multiple medication regimens such as this and are currently doing just that for our on-going trial using bupropion and varenicline. Patients in that trial receive 1 of 2 active medications and a matching placebo of the opposite drug. Thus we have logistical support, expertise and experience to carry out a study using multiple medications and anticipate no problem in successfully conducting these procedures.

Smokers will be instructed to quit smoking the day before Visit 2. We chose the quit date to be consistent with previous trials using these drugs. Current guidelines note that both drugs may be discontinued without tapering. All medication will be stopped at the end of week 12. Subjects' thoughts about their treatment assignment will be assessed by querying participants at the end of treatment, using a brief questionnaire we developed for our current medication trials. While not a valid measure of blinding success, this assessment provides valuable insight regarding the subjects' perception of their medication assignment and the associated side effects (see Appendix X). Medication dispensation, pill counts and assessment of adverse events will be carried out by medical and other research staff members who have been trained on these procedures.

As described above, given the data from Pfizer and the AE profile of both of these drugs, we do not anticipate any significant increase in adverse events as a result of combining these medications. However we will closely monitor AE's during an initial pilot of 5-6 smokers (as well as throughout the study) paying particular attention to the first cohort of patients (approximately 20) to complete the full 12 weeks of combined medication. If the frequency of AE's exceed that which is expected for either drug, we will consider a change in the titration schedule, for example, delaying the onset of bupropion for 1-2 weeks after the start of varenicline, to allow for some adaptation to take place prior to the first drug, before introducing the second medication. The quit date would also be adjusted accordingly. It should also be noted that consistent with the principals of good clinical practice, individual adjustments in medication dose might be occasionally recommended by our study physician at any time, in response to an emergent medical concern. All medication adjustments are recorded in the database and used in the analyses model that includes dose as a covariate (see Data Analysis section below).

Breaking the Blind. Un-blinding of single cases by the investigator will only be performed if relevant for the safety of the participant. The PI along with the Program Director (PD) will be responsible for implementing procedures for maintaining the blind and for breaking the blind when necessary. In emergency situations, the Principal Investigator (PI) would consult with the Program Director (PD) and/or the research team's Data Management Supervisor (DMS) to obtain immediate blinding information for the participant. The PD/DMS would then pass this information on to the PI to enable the participant to be treated. In non-emergency situations, the same procedures would apply, however the PI and PD will discuss and evaluate the request, then, the PI after consulting with the study physician would be responsible for making the decision of whether

or not to un-blind. When the blinding code is broken, the reason will be fully documented and included on the appropriate data collection forms. The MDACC IND office will also be notified.

Un-blinding of all participants will occur at the end of study, whereby the PI will be provided with a list containing data regarding the arms to which each of his patients were randomized. Also, at the end of study, subjects will also be told which medication(s) they were taking if requested.

Counselor Training and Supervision. In order to ensure consistency in the counseling portion of the intervention, all study counselors will be required to attend training sessions with Ph.D. or Master's - level certified smoking cessation clinicians from Dr. Cinciripini team. Dr. Cinciripini and his team are more than qualified to train the study counselors. In addition, many of the clinicians on his team are licensed professional counselors in the state of Texas and have been certified for smoking cessation counseling by a recognized national program at the ACT Center at the University of Mississippi Medical Center which meets the guidelines set forth by the Association for the Treatment of Tobacco Use and Dependence (ATTUD).

Training components include: 1) an overview and rationale for the use of behavioral therapy in smoking cessation treatment; 2) an overview and rationale for the use of the pharmacological treatments used in the study; 3) a comprehensive review of the therapist and patient manuals; 4) watching videos of experienced smoking cessation counselors delivering the counseling intervention, with a follow-up discussion of the techniques used; 5) role play practice of counseling sessions with follow-up discussions; and 6) conducting a counseling session under supervision, again with post-session discussions. All counselors will go through each of these training components at least once and will go through refresher training as often as deemed necessary by the counselor supervisor. In addition, all study counselors meet with the counselor supervisor on a weekly basis (or more frequently if initiated by the counselor) to discuss any patient issues and to get brief training or refreshing in a number of areas relevant to the individual needs/circumstances of the counselor.

Intervention Fidelity. In the proposed study, intervention fidelity will be maintained through several means: manual-driven approaches; on-going training; completion of post-counseling inventory of topic discussed; individual and team supervision; and independent ratings of tape-recorded intervention sessions.

Follow-up Assessments. In person follow-up assessments will be conducted at 3, 6, and 12 months after the target quit date. Similar to the pivotal trials of varenicline, brief telephone contacts at weeks 14, 20, 34, 42 and 48 will be conducted to assess cigarette (abstinence) and other tobacco use as well as use of medications for smoking cessation (See Table 5 for additional details).

Abstinence Assessments. Abstinence outcome assessments will conform to the recently developed guidelines from the Society of Research on Nicotine and Tobacco (SRNT) committee on measurement of abstinence [140], as well guidelines established by the FDA for proof of concept trials of a new smoking cessation medication. The SRNT guidelines recommend measuring and reporting abstinence in several ways, including, point prevalence abstinence, prolonged abstinence, continuous abstinence, and time to relapse. In addition, it is recommended that measures of smoking exposure be obtained to assess reduction among participants who fail to quit. Our analyses will include an evaluation of each of these measures of abstinence as well as smoking reduction.

Seven-day point prevalence abstinence is the most commonly used measure in smoking cessation studies and is defined as a self-report of no smoking, not even a puff, in the 7 days prior to the assessment plus a confirmatory CO <10ppm. Prolonged abstinence refers to abstinence beginning with the end of the post-quit date grace period and extending to a subsequent follow-up point. The grace period is usually 2 weeks but can be any time frame in which the treatment is expected to have produced an effect. For this study, the grace period will be 2 weeks from the quit date. Relapse after the grace period is defined by any smoking (or other tobacco use) over 7 consecutive days or smoking less than 7 consecutive days but over 2 consecutive weeks. Prolonged abstinence will be reported at each time point following the grace period. Continuous abstinence is defined as no smoking, not even a puff, beginning on the quit day through the end of follow-up. Finally, the FDA proof of concept measure is defined as no smoking, not even a puff, for four consecutive weeks during the last 4 weeks of treatment.

Our primary outcome measure for this trial will be prolonged abstinence as that is the recommended SRNT guideline standard. However, to provide comparability to older studies as well as more recent medication trials, we will also report repeated point prevalence, continuous and the FDA proof of concept

measure of abstinence. In addition, our abstinence interview will enable us to capture days to relapse and number of cigarettes smoked at each time point. Days to relapse will be used to conduct a survival analysis, also recommended in the SRNT guidelines. Cotinine will also be assessed at follow-up to provide a measure of tobacco exposure among those who fail to quit. Cotinine levels and self-reported cigarettes per day data obtained through the time line follow-back procedure will be used to evaluate drug related smoking reduction among those who fail to quit.

Participant Compensation Following randomization, participants will be remunerated approximately $20 per weekly clinic visit and $50 for each of the in-person follow-ups for expenses associated with study participation. If a subject completes an EEG assessment, an additional $25 will be offered as compensation. Free parking and/or bus passes (if available) will also be provided.

Design Considerations There are several design issues that were considered in choosing the present methodology. Although the combination of these two drugs has not been extensively evaluated we chose to begin each medication simultaneously based on the fact that a previous short term study of co-administration of both medications (www.chantix.com) showed no significant alteration in the pharmacokinetics of either drug and no significant elevation in side effects. Moreover, although not started together, our preliminary data shows no increase in side effects associated with combined use of these medications. We chose to use the same dose escalation procedure recommended for either drug by itself in order to maximize potential efficacy of the combination. However, it is possible that dose adjustment of either drug in some smokers may be required to medically manage adverse events. This would be consistent with good clinical practice guidelines established for either drug and will be recorded as they occur in the present study.

Similarly, at this stage of evaluation of the combination of these two medications, it seemed inappropriate to include a bupropion alone arm. Two independent studies have already established that varenicline is more effective than bupropion [4,28,6] alone and there are numerous independent trials showing the bupropion out performs placebo[5]. Little seemed to be gained by including a bupropion alone conditions in the current trial since the scientific question at hand is determining whether the combination treatment out performs the solo treatment of varenicline, and to what extent such effects if observed may be mediated by our affective and cognitive measures, relative to placebo. Moreover, given the animal data showing no effect on nAC DA release when nicotine and varenicline are co-administered there appears to be little benefit of including a treatment arm combining NRT and varenicline [24].

We also chose to use a 12 month follow-up period for determining prolonged abstinence (our primary dependent measure) and long term drug effectiveness as we feel this represents an appropriate balance between the definition of abstinence used to establish FDA proof of concept (continuous abstinence over the last 4 weeks of treatment), and the one year continuous outcome milestone most often used as the standard in smoking cessation clinical research. This study, while clearly looking for an off-label application for the combination of both drugs, is not a traditional industry sponsored medication trial. We reasoned that the 4-week proof of concept measure while acceptable for purposes of meeting FDA requirements for moving from a phase 2 to phase 3 medication trials and phase 3 to approval, would not meet the traditional standards for determining abstinence of the research community. The additional cost of extending follow-up to 12 months, in our view, are outweighed by the beneficial contribution of the study to the research literature that would accrue as a function of using a longer follow-up period.

Figure 4

Study Event	*Duration*	*Start*	*End*
Set-up/Hiring and	1	0	1
Database Programming	2	1	3
Staff Training /Pilot	3	3	6
Randomize, Treat, Follow	54	6	60
Complete Follow up & Data Analysisis	6	60	66

Time Line and Pilot. As shown in **Figure 4**, we anticipate conducting a logistical pilot as is our practice prior to full randomization. We will use this time to test the database, assessment procedures, and to complete the real-time training of any new hires. We will run approximately 5-10 smokers through the entire 12 week medication protocol and make any adjustments as necessary in our procedures to facilitate ease of patient flow compliance, and management of side effects. We already have a strong infrastructure in place for conducting clinical trials, including established relationships with medication vendors, our pharmacy and other suppliers, trained medical and support staff, and adequate space and supportive resources to conduct the trial

(see Resources and Environment). Thus we anticipate few problems in getting this study underway. In the time line, we allow for the possibility that completion of the post-treatment phone assessments and 6 and 12 month follow-ups may reach into the 6 month period following the official end date of the grant. We have budgeted our resources from this project to allow for the needed staff to complete these follow-ups in a no-cost extension period should that be necessary.

Data Analytic Plan

Prior to inferential procedures, extensive descriptive analyses will be conducted on the screening/baseline data and repeated measures data from study questionnaires, medical assessments (including laboratory tests, dosage levels, pill counts, etc.) and adverse event reports (see Table 5). For continuous measures standard descriptive statistics, including means, standard deviations, ranges, etc., will be computed together with ninety-five percent confidence intervals. Graphical methods, including box plots and histograms, will also be employed to closely examine the distributions of the questionnaire scores. If required, potential normalizing transformations will be explored. Vicariate associations between the scores and selected demographic variables including age, ethnicity, gender, initial cotinine, FTND score, depression and ADHD history, baseline smoking level and other initial assessments will be evaluated using Pearson's product moment correlation coefficients and ANOVA. Cross-tabulations and chi-square analyses will be used to examine questionnaire categorical data and relations with study group.

General Statistical Approach.

Logistic regression will be used to evaluate the prolonged abstinence at 12 months (primary outcome) as well as exploratory abstinence outcomes, including EOT and 6 months abstinence and all other abstinence definitions at each time-point. The primary contrast of interest compared the Combo and Var groups. Missing abstinence observations will be imputed as smoking, Moreover, our analytic strategy will involvethe use of a mixed model regression to examine the effects of the dependent variables (e.g., abstinence, PANAS, WSWS, mCEQ, CAARS, cognitive assessment tasks, etc.) across assessments. The mixed model approach provides a generalization to the classic linear regression model, using likelihood functions instead of least squares to estimate effects [141]. The mixed model approach is ideally suited for analysis of repeated measures data in that it allows for more specific estimation of the correlation structure of the residuals, and more efficiently handles unbalanced designs and is very robust to missing data, without excluding participants or imputing values [142,143]. Fit statistics (e.g., Akanke's Information Criterion) will be evaluated for all models to ascertain the best fit of the correlation structure of the dataset. We will use a computer program, PROC MIXED (SAS v9.1 SAS Institute Inc, Cary, NC) to estimate and to test the models with continuous dependent measures. In the case of abstinence data where the value is binary, we will use SAS PROC GLMIMX, which provides an adaptation to the mixed model approach for categorical data. Post hoc comparisons within multilevel main effects and between interaction terms will be conducted using tests of simple main effects [144]. We have considerable experience with these procedures and have employed them in our previously published studies [145,23,146]. Post hoc comparisons within multilevel main effects and between interaction terms will be conducted using tests of simple main effects [144]. We have considerable experience with these procedures and have employed them in our previously published studies [145,23,146].

EEG Statistical Approach. To test our exploratory hypotheses involving EEG, the information from the whole topographies will be used. To avoid setting arbitrary regions of interests and to take advantage of the better spatial resolution provided by the dense-sensor array, we will perform the statistical analyses using randomization tests to control for the increase of the family-wise error rate due to multiple comparisons [133]. The randomization procedure involves two steps: (a) computing a statistic (e.g., an F-statistic) for each sensor and (b) evaluating its p value under the randomization distribution. The randomization distribution is built by first randomly assigning to different data vectors the data matrix obtained for each participant within each experimental condition. The statistic of interest is then calculated for each sensor. These are the F-values obtained when the data are randomly assigned to the experimental conditions and the highest value is stored. This process is repeated 10000 times to form a distribution for the F-statistic associated with the hypothesis of interest. After the construction of these randomly generated data vectors, the F-statistic is computed at each sensor using the actual data as obtained from the experiment. If the value of the F-statistic obtained during actual hypothesis testing exceeds the F-value marking the upper 5% of the distribution obtained from the random iterations, then it is considered significant at the .05 level. To test each hypothesis a new

randomization distribution will be generated. This will ensure the independence of each statistical test and will keep the probability of a Type I error within the 5 % threshold for each comparison. An example of our work and approach in this area can be found in Appendix T.

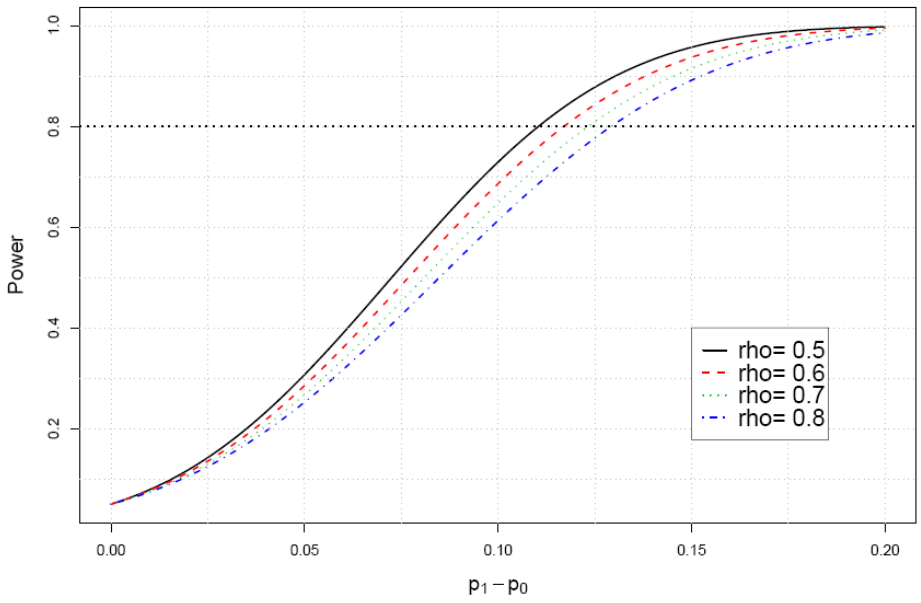
Power Analysis. Our primary hypothesis is that abstinence rates will be significantly greater in VB vs. the V group at the end of treatment and through the follow-up assessments. Based on our previous experience using monetary compensation for completion of assessments, we expect the follow-up completion rate to be approximately 80%. Intention-to-treat will be used as the standard for all analyses, classifying participants whom we are unable to contact at follow-up as smokers, thus no abstinence data is regarded as missing once a participant has been randomized. Because of the binary form of the primary outcome measure, the multiple follow-up visits and the potential for non-homogeneous correlations among the measures over time, we have estimated power across the time points (EOT-through 1 year follow-up) using power calculations for longitudinal data with binary responses [147], assuming that we will have an equal number of study participants in each treatment group from the intent to treat analysis, and that the impact of the effect of the other covariates will be equal across treatment groups.

As the combination of varenicline and bupropion has not been used in a smoking cessation trial, there are no data from which to directly estimate effect sizes of the combined treatment. However, data from the pivotal trials with varenicline provide a basis from which we can estimate needed power in order to detect reasonable and meaningful differences in abstinence between varenicline alone and the combined treatment. In these trials both 7-day point prevalence and continuous abstinence are reported for the end of treatment (week 12) and both 6 and 12 month follow-up points. Estimates of prolonged abstinence using the SRNT guidelines, which will serve as the primary dependent measure in this study, were not provided. It is expected that prolonged abstinence rates could be somewhat higher than continuous abstinence rates given the more liberal definition of relapse. Nevertheless, for purposes of calculating power we chose to use continuous abstinence at the selected time points, as this may provide the more conservative estimate and is preferred over 7-day point prevalence for reporting abstinence in clinical trials. In the two pivotal varenicline trials continuous abstinence at the end of treatment, and the 6 and 12 month follow-up were, 44%, 30% and 23% [4] and 44%, 30% and 22%[6] for the two respective trials. Our analysis will involve one additional time point, 3 months post quit, which lies between the EOT and 6 month follow-up in the pivotal studies. For purposes of power calculations we took the average abstinence between the end of treatment and the 6 month follow-up point to provide one additional point to be used in the calculations. In order to calculate power for our study, we estimated the average varenicline abstinence rate across these time points to be 33%.

Power curves for abstinence are depicted in **Figure 5** for several possible intra-class correlations (rho), assuming at least 150 subjects per treatment group and a Type I error rate of 0.05. Even assuming a conservative intra-class correlation of 0.8, our power analysis indicates that our sample size provides 80% power to detect about a 12% increase in abstinence or an odds ratio of 1.67 or greater, for the combined treatment.

Our preliminary data may also provide an estimate of expected abstinence rates in the combined group of those who would otherwise remain as treatment failures if not treated with the combination therapy. Based on an observed 7 day point prevalence rate of abstinence of 50.3% at the end of treatment in the pivotal trials and a 27.3% abstinence rate among those who received combination therapy in our preliminary study (after initially failing to quit), we calculate an expected effect size as follows: .503 + (1- .503)*.273= .64; .64-.503=.14 or 14% improvement in abstinence rates among nonabstainers. In this case, given that the 7 day point prevalence abstinence rates for varenicline alone at the end of treatment was 50.3% that could mean the overall success rate in the combined group could reach 64%. We cannot determine from our data how this difference will hold up over time but most smoking cessation data (our own included from multiple trials) suggest that the percentage differences between treatment and controls tend to remain constant from the end of treatment through follow-up, although the absolute cessation rates decrease. The average effect from the end of treatment through follow-up could be 10-18%, allowing a 4% difference on either side. This is likely at the very low end of such estimates given the fact that it is based on a difficult to treat population and due to the fact that the combined intervention was instituted later (after 8 weeks of monotherapy) rather than earlier in our pilot. These figures could underestimate of the number of early treatment failures we might have captured had we intervened sooner. For example, smokers who fail to quit may become discouraged, stop trying and

Figure 5 Power Curves for Abstinence Assessments



withdraw from treatment. Combination therapy from the starts may reduce the likelihood of this happening. Our choice to power our study for an average 12% difference seems reasonable in this regard and provides for a meaningful difference between V and VB groups.

Data from our own smoking cessation studies on nearly 1000 smokers [148,23] suggest that the intra-class correlation coefficient for point prevalence and continuous abstinence across multiple post quit assessment points may be considerably lower than .8 (range .4-.6), which would favorably impact power, and afford detection of smaller differences in abstinence between the two groups, given the same sample size. Thus, we expect power to be adequate for detecting differences of at least 12% which would represent a meaningful increment in efficacy of the combined treatment. Although not our primary outcome measure, we also estimated power for detecting differences in continuous abstinence during the last 4 weeks of treatment (FDA proof of concept measure) and at the one year follow-up. Given the data for these time points in the studies cited above, we will have 80% power to detect differences of 16% at the end of treatment; and 12.5% at the one year follow-up, for a comparison between the V and VB groups. All comparisons with placebo, while not part of our primary predictions are adequately powered since the difference between P and either active treatment is likely to be substantially larger than the 12% between the two active groups (i.e., based on data from the pivotal trials the difference between P and V at the end of treatment was approximately 27%).

We also estimated power to detect differences between the groups in measures of negative affect and smoking reinforcement (we used the satisfaction scale from the mCEQ for this purpose) as these are among our primary measures secondary to abstinence. Means and standard deviations of negative affect and smoking satisfaction scores for varenicline were taken from the Gonzales study (means were not reported in the Jorenby study, just difference scores). These trials used the Minnesota Withdrawal scale to measure negative affect and the mCEQ to measure smoking satisfaction. We are using the WSWS and the PANAS as primary measures of negative affect. These scales have superior psychometric properties in relation to the MNWS and were conceptualized and developed for research specifically evaluating the role of negative affect in smoking withdrawal in the case of the WSWS; and the circumplex theory of emotion in the case of the PANAS. Although scored for other subscales, the WSWS has within it, the identical items that comprise the MNWS so a comparison can be made to MSWS scores from other studies. For purposes of calculating power, we used data from the Gonzales study to estimate mean MNWS negative affect and mCEQ scores for varenicline; and data from our own clinical trials to estimate PANAS and WSWS scores, since these scales were not given in that study. With a sample size of at least 150 per group, we would have 80% power to detect differences effect size of 0.21-0.26 standard deviations for the MNWS/PANAS/WSWS/mCEQ, across subscales, assuming a value of rho ranging from .4-.8 among four repeated measures. The power calculation is based on the method provided by Diggle and colleagues [147]. Thus we should have enough power to detect small to moderate effect size differences between groups given the current sample size.

Adjustment of Sample Size Addendum on 2/1/2013. We are requesting approval to increase the total number of subjects in this protocol from 350 to 385, an increase of 35 overall participants. Our original estimate for a sample size of 350 was based on a 20% attrition rate. We recently examined the blinded data and noted

the attrition rates (combined lost to follow-up and withdrawal) averaged 30%. This should not affect our primary analysis for abstinence since we will be carrying out an intent to treat analysis for smoking abstinence, in which missing data is assumed to be smoking. However, the accuracy of our estimates for our continuous secondary measures, such as nicotine withdrawal, might be reduced. Based on the original sample of 350, about 70 subjects might be expected to have missing data at one or more of the time points for the analysis of withdrawal. Using the attrition rate of 30% noted above this could rise to 105, a difference of 35 participants. Our randomization is based on a 3:3:1 ratio for varenicline, varenicline plus bupropion, and placebo (adding an additional 15:15:5 subjects). We have also taken other steps noted elsewhere in the protocol to enhance our follow-up rates, and to capture the missing data on current participants.

Our original calculations on ITT analysis allowed us to detect a 12% difference. We originally estimated based on our preliminary data that the treatment difference could range between 10% and 18%. With the increase in subjects, we still expect the treatment difference to be in this range. With this increase in subjects, our power to detect treatment difference will remain above 80%. We also expect that the increase in sample size will compensate for the higher than expected attrition rate and will improve the accuracy of our estimates for our continuous secondary measures to the level that we had expected originally.

Statistical Analyses of Primary and Exploratory Aims

Primary Aim & Hypotheses

1. To evaluate the efficacy of varenicline plus bupropion (VB) vs. varenicline (V) or Placebo (P) alone for smoking cessation,
   1.1. We hypothesize that smokers treated with the combination therapy will be abstinent significantly more often and take a longer time to relapse at 12 months follow-up than those treated with varenicline alone.
      1.1.1. Logistic regression analysis will be used to evaluate this hypothesis, regressing prolonged abstinence at 12 months follow-up on Group (Varenicline/Varenicline plus Bupropion/Placebo). Prolonged abstinence is defined as no smoking from the quit date to the 12-month follow-up, verified by expired CO < 4 ppm. As part of the exploratory analyses, we will also evaluate 7-day repeated point prevalence abstinence, continuous abstinence (single value-no repeated measure) from the quit date through the 6 and 12 month follow-ups as well as 4-week continuous abstinence from the end of treatment (FDA proof of concept measure). These measures are described in the “Abstinence Assessment” section in methods.

Exploratory Aim & Hypotheses

1. To evaluate the effects of VB vs. V and P on measures of nicotine withdrawal, negative affect, depression, smoking reinforcement, sleep problems, and craving, and measures of cognitive performance.
   1.1. We hypothesize that smokers treated with the combination therapy will report significantly lower levels of nicotine withdrawal symptoms, negative affect, smoking reinforcement, sleep problems, and craving over the course of treatment, and improved cognitive performance during quitting, than those treated with varenicline alone.
      1.1.1. Mixed model repeated measure regression analyses will be used to evaluate this hypothesis, regressing scores from the negative and positive affect scales of the PANAS, total depression from the CES-D, total score from the Sleep Problems Scale, subscales of the WSWS (Anger, Anxiety, Concentration, Sadness and Craving), QSU, and mCEQ (Smoking Satisfaction, Psychological Reward, Enjoyment of Respiratory Tract Sensations, Craving Reduction and Aversion), scores on the Conners Adult ADHD rating scale(CAARS), and each of the cognitive assessments (ANT, RVIP-CED), on Group (VB/V) with Time as the repeated measures factor. The main effect of treatment Group is of primary interest in this model and will be evaluated first, followed by Time, and the Group X Time interaction. In this model, the pre-medication/pre-cessation value of the dependent measure from Visit 1 will serve as the covariate. In addition, we will also test models that include abstinence status at each time point as a covariate to control for the effects of smoking (among relapsers) on negative affect and withdrawal.

1.1.2. We will also evaluate the extent to which changes in these process variables (e.g., affect, cognitive function, smoking satisfaction, etc.) on each of the process measures described above in 2.1.1may mediate the effect of treatment group on abstinence. For this analysis we will use end of treatment abstinence rates as they are the most proximal to the time the process measures are obtained. Our group has the requisite experience conducting meditational analyses and has published two papers in this area: one using the procedures of Baron and Kenny [149], examining mediators of the effects of depression history on abstinence [150]; and those of MacKinnon [151] estimating meditational effects of psychological process variables in cognitive behavior therapy [152]. We will use the later approach to examine the extent to which our major process variables may account for differences in treatment outcome. MacKinnon's approach is very straight forward and involves using a series of regression analyses to determine the effect of the independent variable (treatment) on outcome (abstinence-as we propose to do to test our main hypotheses), and to use this same approach to determine the effect of the independent variable on the hypothesized mediator (e.g., PANAS negative affect scores; CES-D scores). Mediation is determined by examining the extent to which including the hypothesized mediator in the regression model predicting outcome along with the independent variable, changes that relationship. This can be calculated by dividing the mediated effect by its standard error resulting in a z-score which can be evaluated for significance [153]. Guidelines we have followed in our previous work can be found on Dr. MacKinnon's website (see http://www.public.asu.edu/~davidpm/ripl/mediate.htm#whatis). For our initial analysis we would examine the mean of the hypothesized mediators in the first and second week post-quit, since this time frame for examining withdrawal and affective states has been shown to be predictive of relapse in earlier studies [94,68,154]. We would also evaluate longitudinal models where the repeated assessments of the mediator allow us to determine whether or not changes in the mediator occur prior to the subsequent measure of outcome (i.e., is a reduction in withdrawal from baseline for example, on the quit date or at subsequent times associated with end of treatment abstinence between the groups. We will use a series of regression analyses as outlined by MacKinnon [155].

2. To evaluate the effects of VB vs. V on lapse progression
   2.1. This is an exploratory aim to evaluate possible differences between the two treatments on time to an initial lapse; and time between an initial lapse and relapse. This later analysis will involve smokers who achieve initial abstinence but who are not continuously abstinent during treatment.-Little is known about this group of smokers given the concentration of pharmaceutical research on treatment efficacy involving smokers who are continuously abstinent. We will test for the possibility that VB is more beneficial than V on preventing a lapse from resulting in a relapse.
      2.1.1. For this analysis we will follow the procedures outline by Shiffman and colleagues [156] who evaluated the effects of NRT on lapse progression. We will use several treatment milestones and construct survival curves using Cox proportional hazard, for the percent of individuals within each group that achieve that milestone as a function of time. The milestones are as follows: 1) time to achieve initial abstinence (abstain for 24 hours) beginning on the target quit date; 2) Days to lapse (first smoking event after achieving initial abstinence); 3) Days to relapse after the first lapse. For this analysis we will construct models using 2 SRNT definitions of relapse (i.e., 7 consecutive days of smoking or smoking over 2 consecutive weeks.
      2.1.2. As recommended by the SRNT guidelines, a survival analysis will also be carried on days to relapse using Cox proportional hazards regression.
3. To evaluate the effects of VB vs. V and P on smoking reduction among those who fail to quit.
   3.1. We hypothesize that non-abstinent smokers treated with VB will smoke significantly less than smokers treated with V.
      3.1.1. Mixed model repeated measure regression analyses will be used to evaluate this hypothesis, regressing average daily cigarette consumption (from the abstinence interview) and cotinine values on Group (VB/V/P) with Time (e.g., Visits 2-16 for cigarettes and 2, and

13-16 for cotinine) as the repeated measures factor. The main effect of treatment Group is of primary interest in this model and will be evaluated first, followed by Time, and the Group X Time interaction. In this model, the pre-medication/pre-cessation value of the dependent measure from Visit 1 will serve as the covariate.

Each of the models described above will be run with and without covariates and we will report on any differences in models with and without covariates. Given our careful balancing strategy in group assignment we do not expect major differences in factors previously associated with difference in treatment out come (e.g., FTND, depression history) For the mixed models, the likelihood ratio test procedure will be utilized to compare two models where one (without covariates) is a special case (with covariates) of the other. The covariates to be used include ethnicity, age, gender, smoking history, and depression history. Interactions between ethnicity or gender and treatment will be explored. If we find statistically significant interactions, separate models for each will also be tested. We will also test models that include medication levels, average dose, and visit compliance and dose covariates based on biological assays, pill count, and sessions attended, respectively. Although the results of the analyses with these covariates are secondary to our main intent to treat models described above, they will provide information regarding the relationship between compliance with pharmacological and behavioral treatment recommendations and overall treatment outcome.

Interim Analysis

In this trial, the focus of the interim analysis is toxicity. The efficacy of Varenicline and Bupropion in improving smoking cessation has been very established[7,8] and both of these medications have been FDA approved for smoking cessation. We do not expect the early stopping due to futility. The side effect profiles of Varenicline and Bupropion have been well characterized. From a clinical standpoint the most potentially significant side effect profile would involve neuropsychiatric symptoms, such as depression, anxiety, suicidal ideation. This protocol includes a specific plan to monitor neuropsychiatric events and intervene as required. Risks for these events are also reduced by the exclusion of participants with a current psychiatric disorder as defined by out standardized screening instruments (i.e., MINI). Nevertheless we consider the occurrence of these symptoms at a Grade III level as the basis for developing a stopping rule for this study.

Figure 6. Adverse Events for Varenicline and Bupropion

	Varenicline (n = 343)	Bupropion SR (n = 340)	Placebo (n = 340)
Nausea	101 (29.4)	25 (7.4)	33 (9.7)
Constipation	31 (9.0)	22 (6.5)	5 (1.5)
Flatulence	20 (5.8)	7 (2.1)	8 (2.4)
Dry mouth	19 (5.5)	26 (7.6)	11 (3.2)
Dyspepsia	19 (5.5)	10 (2.9)	12 (3.5)
Vomiting	18 (5.2)	7 (2.1)	6 (1.8)
Insomnia	49 (14.3)	72 (21.2)	42 (12.4)
Abnormal dreams†	45 (13.1)	20 (5.9)	12 (3.5)
Sleep disorder	16 (4.7)	23 (6.8)	9 (2.6)
Anxiety	15 (4.4)	18 (5.3)	13 (3.8)
Headache	44 (12.8)	27 (7.9)	43 (12.6)
Dizziness	22 (6.4)	25 (7.4)	24 (7.1)
Fatigue	25 (7.3)	13 (3.8)	22 (6.5)

Abbreviation: bupropion SR, sustained-release bupropion.
*Adverse events occurred at a rate of 5% or higher in participants receiving varenicline or bupropion SR compared with participants receiving placebo. These adverse events began or increased in severity during treatment or up to 7 days after the last dose.
†Self-described by the participants as any change in dreaming, such as vivid dreams or increased frequency of dreaming.

Ongoing assessment of these symptoms will be accomplished initially through the collection of adverse events at each visit. Participants will be asked open-ended questions regarding any changes to their physical or mental health since the previous visit. This is our standard practice. In the event that a patient reports any severe psychiatric symptom, an in-depth clinical assessment will be initiated. Patients will be evaluated for severe mood, anxiety and psychotic symptoms (encompassing symptoms such as irritability and agitation) using version 5.0 of the MINI International Neuropsychiatric Interview. Any patient who meets criteria for a severe psychiatric disorder on the MINI will be considered as a case to be included in the stopping rule for that disorder. Severe in this context is defined as marked limitation in activity that interrupts the subject's usual daily activity and requires medical intervention/therapy and/or hospitalization. All individuals who meet criteria for a severe psychiatric disorder will be referred to the Clinical Psychologist and/or Addiction Psychiatrist for further assessment and clinical management.

If 5 serious neuropsychiatric events occur at anytime during the trial we will take steps to halt recruitment and examine the un-blinded data to determine if the rate of these events in the combination arm (varenicline plus bupropion) differ significantly from what is observed in the placebo group. A serious

neuropsychiatric event will be defined as those which meet the regulatory definition of serious adverse event as well as those defined by a marked limitation in activity that interrupts the subject's usual daily activity and requires medical intervention/therapy and/or hospitalization. After an examination of the unblinded data by the PI, medical staff and study statistician a determination will be made to continue if the rates of these events in the combination arm do not significantly exceed that observed in the placebo group.

E. Human Subjects Research - Protection of Human Subjects

Risks to the Subjects

Human Subjects Involvement and Characteristics: Subjects recruited for this study (N=385) will be current smokers from the Houston metropolitan community. Based upon our past experiences recruiting from this population, we estimate that we will need to screen up to 1100 people in order to attain the targeted 385 eligible participants who will actually be enrolled in the study. Inclusion criteria are presented in Table 4**.** All smokers meeting these qualifications will be accepted into the study. Given the nature of the study design it will be necessary to eliminate subjects who do not speak English or have a telephone.

Sources of Materials: Participants will be providing physiological data in the form of blood pressure, weight, blood chemistry, and saliva cotinine. Questionnaire data will be obtained that assess previous smoking, smoking cessation history (including Rx medications and NRT's) and health history (including depression and ADHD), mood, nicotine withdrawal, craving, and cigarettes smoked. All data will be collected specifically for research purposes and will be coded to maintain confidentiality. Screening laboratory data will be shared with the participant and appropriate referral for follow-up medical care will be provided as needed.

Potential Risks: As reported in Micromedex (Thompson Healthcare) the frequency of adverse events reported for varenicline are as follows: Abdominal pain (5% to 7%), Constipation (5% to 8%), Flatulence (6% to 9%), Nausea (16% to 40%), Vomiting (1% to 5%); Neurologic: Dream disorder (9% to 13%), Headache (15% to 19%), Insomnia (18% to 19%). The typical side effects are not usually serious in nature and often abate within a few days to weeks after starting medication or once the medication is withdrawn. The most common, nausea, was rated by the majority of patients who experienced it as mild to moderate and resolved after 10-12 days. Less than 3% of smokers in the pivotal trials withdrew due to nausea.

The frequency of adverse events reported for bupropion are as follows: Hypertension (2% to 4%), Tachyarrhythmia (11%); Pruritus (2% to 4%), Rash (3% to 5%), Urticaria (1% to 2%); Weight change (3% to 23%); Constipation (5% to 10%), Disorder of taste (2% to 4%), Nausea (13% to 24%), Pharyngitis (3% to 11%), Xerostomia (17% to 26%); Arthralgia (1% to 4%), Myalgia (2% to 6%); Confusion (8%), Dizziness (6% to 11%), Headache (25% to 34%), Insomnia (11% to 20%), Tremor (3% to 6%); Tinnitus (3%) Psychiatric: Agitation (2% to 9%), Anxiety (5% to 7%), Hostile behavior (6%); Disorder of menstruation (5%); Cardiac dysrhythmia (5%); Stevens-Johnson syndrome (rare); Anaphylaxis; Seizure (0.1% to 0.4%); Depression, exacerbation, Mania, Psychotic disorder, Suicidal thoughts.

Because bupropion is also used as an antidepressant with smokers and non-smokers and often used far longer than proposed here it is informative to assess the specific adverse events profile on a population of smokers trying to quit. Data from the pivotal trials using varenicline provide an AE frequency table for both bupropion and varenicline [6], as shown in Figure 6

Post-Marketing Experience: The following adverse events have been reported during post-approval use of varenicline. Because these events are reported voluntarily from a population of uncertain size, it is not always possible to reliably estimate their frequency or establish a causal relationship to drug exposure. There have been reports of depressed mood, agitation, changes in behavior, suicidal ideation and suicide in patients attempting to quit smoking while taking varenicline. Smoking cessation with or without treatment

is associated with nicotine withdrawal symptoms and the exacerbation of underlying psychiatric illness. Not all patients had known pre-existing psychiatric illness and not all had discontinued smoking. The role of varenicline in these reports is not known. Participants are informed of these issues in the informed consent form. In addition, they are told that should they experience any of these symptoms, to stop taking the medication and call our study physician immediately. We have also developed a comprehensive plan to monitor and evaluate any emergent psychiatric symptoms, including depression, anxiety and suicidal ideation.

Participants may also experience minor bruising, pain or infection following phlebotomy, skin irritation due to electrode adhesive for an ECG assessment (if required), and tobacco withdrawal effects (e.g., increased irritability, difficulty concentrating, etc.) during smoking cessation. None of these effects typically result in serious adverse health consequences. It is unlikely that completing questionnaires would lead to any potential risks for participants. In sum, it is highly unlikely that any legal, social, or psychological problems will result from this research.

Adequacy of Protection Against Risks

Recruitment and Informed Consent: Participants will be recruited from the Houston community sample using: (1) mail, public service announcements, media interviews, MD Anderson Internet access, newspaper advertisements, MD Anderson Conquest Magazine; (2) through the MDACC community liaison and outreach offices, sending advertisements and mailers to all affiliated providers on the mailing list. Consent will be obtained at the onset of the orientation/baseline interview. Participants will be provided with a detailed description of the study, information on risks, and on their right to withdraw from the study.

Protection against Risks: Varenicline and bupropion are both FDA approved medications for smoking cessation. We will follow the medication dosing procedures used for both drugs in the original clinical trials comparing varenicline and bupropion to placebo. Moreover, we will also provide smoking cessation counseling consistent with the Clinical Practice Guidelines for Smoking Cessation. Our procedures closely follow those used in previous clinical trials involving several medications for smoking cessation. Our study physician will identify participants who have contraindications for use of either drug and we will monitor participants for adverse reactions while they are on medication. The risks of blood sampling will be minimized by use of trained phlebotomists and the provision for on-site medical assistance should any untoward complications result. The Tobacco Research & Treatment Program (TRTP) clinic is located adjacent to the Department of Clinical Cancer Prevention, which has trained medical personnel on staff that will be available to assist the study physician and other personnel in managing medically related study issues. Confidentiality will be protected by identifying all subjects by ID numbers in all data used outside the institution (e.g., laboratory assessments). Analyses of such data are provided by sample number coded on each collection container and cannot be connected to individual participant names by the laboratory conducting the assays. Only the PI and his staff will have access to the master file linking laboratory and other data to participant names. All study data files are server maintained with limited access using password entry and log in restrictions to study staff. All information will be reported in aggregate form and individual participants will not be identified in any public reports or documents. We expect these procedures to be highly effective for protecting participant confidentiality. See Data and Safety Monitoring section for further details concerning protection against risks.

Potential Benefits of the Proposed Research to the Subjects and Others

A primary benefit to participants in the proposed study is smoking cessation. All participants will receive an empirically validated treatment for smoking cessation (smoking cessation counseling) and two groups will receive active medication. We anticipate that many of them will continue to be non-smokers after the completion of the study. Smoking cessation is important in cancer prevention, cardiovascular events and emphysema rate reduction therefore reducing medical costs, and increasing well-being for both the participants and society in general. Smoking cessation is cost effective and results in a substantial reduction in healthcare costs for both the individual and society.

Importance of the Knowledge to be Gained

The potential to add a new medication to the treatment armamentarium allows for expanded future research on the assessment of individual differences in patient characteristics that may predict drug response, including genetic factors related to dependence, metabolism and psychiatric risk factors. The ultimate goal of such studies will be to eventually develop tailored treatments that maximize an individual's chances at success by pairing treating them with the correct compound(s). Given the significant benefits that would accrue with increased effectiveness in smoking cessation, these potential benefits far outweigh the risks associated with the proposed research.

Inclusion of Women

Women participants will be included in this research and will comprise approximately 50% of the population sample. In our previous research we have encountered no difficulty in the recruitment of women participants.

Inclusion of Minorities

The population of the Houston community from which the sample will be drawn (includes Harris county) is estimated at 3,596,086 people. The ethnic distribution has been reported as 59% Caucasian (42% of which are not of Hispanic origin) ;19% African-American; 5% Asian; and .4% Native American, with 33% Hispanic or Latino (of any race) [112]. We expect to recruit minority smokers in proportion to the population demographics. We have had good success recruiting from ethnic minority populations, especially African Americans across all of our studies. Our success with Hispanic smokers has been more modest although it must be noted that smoking rates are lower in the Hispanic and Latino community in comparison to the non-Hispanic community. We expect to attract minority smokers to the proposed study using direct public service advertisements targeted for minority smokers on Houston radio stations and newspapers supporting a large minority audience. Houston has two television stations, and several radio stations and newspapers that serve the Hispanic Community. The office of Public Affairs at MD Anderson has also agreed to assist us by arranging for our participation in institution wide Cancer Prevention outreach programs directed at the Hispanic Community. Such events are sponsored several times a year in areas of the community with high concentrations proportions of minority Houstonians. We will focus additional recruitment effort on these venues to increase our recruitment of Hispanic smokers. Such efforts will be in addition to the normal interviews, advertisements, and news releases conducted on our behalf by the Office of Public Affairs at MDACC. Recruitment and enrollment for the proposed study is scheduled to begin as soon as (Institutional Review Board) IRB approval is obtained (within 3-5 months of funding).

Inclusion of Children

We will exclude smokers under the age of 25 because the focus of our intervention is on adult smokers. Additionally, individuals 24 years of age and younger have a higher risk of suicidal tendencies with some of the study drugs. The characteristics of smokers seeking treatment in smoking cessation trials have been very consistent in our recruitment as well as national samples. The average age of these smokers is over 40; they consume about a pack of cigarettes or less per day, have made numerous quit attempts, and have smoked for over 15 years ([23]. There are also likely to be significant differences between adults and adolescent in numerous domains including physiological (e.g., physiological responses to nicotine may be different in adolescents), and psychological (e.g., developmental processes may affect mood self-reports). Therefore, the study of the cessation and emotional processes related to smoking behavior among adolescent smokers requires a separate focus on those factors that are relevant for this population.

DATA AND SAFETY MONITORING PLAN

The IRB of the University of Texas M. D. Anderson Cancer Center (MDACC) reviews and approves the Data and Safety Monitoring Plan for all clinical trials This study will be monitored by the institutional DMC as determined by the IRB during their review of the protocol. Plans and procedures for maintaining data

integrity, defining and reporting adverse events/experiences, and IRB oversight and monitoring of this project are described below. These procedures include monitoring of participant eligibility and accrual, adverse events, interim data analyses, etc. The procedures for monitoring by the IRB, PI and DMC are described in separate sections below, followed by a section defining and further describing procedures for reporting adverse experiences as well as procedures for ensuring data quality and integrity.

IRB Monitoring

During the protocol review and approval process, the IRB determines the level of safety monitoring required for each protocol. The minimum monitoring requirements include investigator monitoring of participant safety, adverse event (AE) reporting in compliance with IRB, NIH, and FDA guidelines, and participation in the Continuing Review process with the IRB. Clinical trials may also be monitored by the Data Monitoring Committee (DMC). The outcomes of IRB and DMC reviews are conveyed to the PI via the administrative support staff in the Office of Protocol Research (OPR). As a medication related clinical trial this study will be monitored by the institutional DMC.

For all protocols conducted at the MDACC, the PI is responsible for submitting Adverse Events AE's to the IRB. The MDACC's policy for AE submission has been defined and approved by the IRB and must be included as an appendix to all protocols. AE's are submitted to OPR and forwarded to the designated IRB vice chairperson for review. Attached to each AE, is a listing of all prior AE's submitted for that protocol. Any comments, questions or changes the IRB requests to the protocol as a result of this review are conveyed to the PI. The PI response and protocol modification process is monitored by the IRB vice chairperson and OPR support staff. The vice chairperson presents the report on AE review to the full committee at the next IRB meeting.

All protocol participants will be registered in the Protocol Data Management System/Clinical Oncology Research System (PDMS/CORe) according to institutional guidelines. All other study-related data, including adverse events, will be entered into a database designed and maintained by the PI and his data management team. Representatives from The Office of Protocol Research will have unrestricted access to the database for auditing and other regulatory purposes.

Table 7. Targeted/Planned Enrollment Table

Study Title: Effects of Varenicline and Bupropion on Smoking Cessation and Nicotine Withdrawal

Total Planned Enrollment: 385

TARGETED/PLANNED ENROLLMENT: Number of Subjects			
Ethnic Category	**Sex/Gender**		
	Females	**Males**	**Total**
Hispanic or Latino	**31**	**46**	**77**
Not Hispanic or Latino	**154**	**154**	**308**
Ethnic Category Total of All Subjects*	**185**	**200**	**385**
Racial Categories			
American Indian/Alaska Native	**0**	**0**	**0**
Asian	**3**	**6**	**9**
Native Hawaiian or Other Pacific Islander	**0**	**0**	**0**
Black or African American	**46**	**58**	**104**
White	**136**	**136**	**272**
Racial Categories: Total of All Subjects *	**185**	**200**	**385**

Monitoring by the Data and Safety Monitoring Board

If required by the IRB the protocol will receive monitoring by the (Data Monitoring Committee) DMC. The DMC is an officially constituted committee of MDACC designed to oversee the data and safety monitoring of clinical trials. The primary objectives of the DMC are to:

- ensure that participants in a trial are protected;
- ensure that participants' interests are not made secondary to the interests of the scientific investigation; and
- monitor all clinical trials that originate at MDACC or that are coordinated or analyzed by the MDACC.

The DMC has the following responsibilities to accomplish the above objectives:

- to review interim analyses of outcome data (prepared by the study Statistician or other responsible person at the time points defined in the protocols approved by the IRB), and to recommend, if necessary, whether the study needs to be changed or terminated based on these analyses;

- to determine whether and to whom outcome results should be released prior to the reporting of study results from this trial at the time specified in the protocol;

- to review interim toxicity data and efficacy of treatment;

- to review major modifications to the study proposed by the PI prior to implementation (e.g., termination, dropping an arm based on toxicity results from this trial or results of other trials, increasing target sample size);

- to communicate information and recommendations to appropriate persons at MDACC regarding the assessment of issues or problems and effective resolutions for educational purposes and improved participant care and risk prevention.

The Committee consists of not more than 15 members (including the Chairman). A majority of members attending meetings of the DMC constitute a quorum. Appointments are made based on the breadth of backgrounds and experience. The committee includes scientists and statisticians from within and outside the institution selected based on their experience, reputation for objectivity, absence of conflicts of interest, and knowledge of good clinical trial methodology. At least fifty one percent of the voting members are not affiliated with MDACC. DMC members represent participant interests, and not that of the institution.

The DMC meets at least once a year and more often if necessary. Each randomized clinical trial protocol has specified interim analyses times. Information provided to the DMC include: title of study, PI, date start of study, expected termination date, expected total number of participants, number of participants entered currently, data from interim analyses, date of interim analyses, toxicity concerns, and the next formal monitoring date as specified in the protocol. The PI may prepare a report addressing specific toxicity concerns or other concerns about the conduct of the study during the open session. A copy of the statistician's report may be sent to the DMC Chair for presentation during the closed portion, but not to any other individuals not on the DMC. The report may contain recommendations on whether to close the study, whether to report the results, whether to continue accrual or follow-up and whether DMC discussion is needed.

The review of each trial may include two parts. The first part will be an open session in which members of the study team may be present at the request of the DMC to answer questions. In this part, the focus is on accrual, compliance and toxicity issues. Following this open session, there will be a closed, executive session in which the DMC discusses interim outcome results by treatment arm, what action needs to be taken, and then votes. At the executive session, those present are limited, to DMC members, alternates, and ex officio members. If a decision is made by the DMC to modify or discontinue a trial - recommendations will be made as to whether and how participants are to be informed and by whom and communicated to the PI in writing. Copies of such communication will be preserved in the official Committee Minutes.

DMC recommendations are based upon results for the current study being monitored as well as upon data available to the DMC from other related studies. The PI will assure that the DMC is advised about relevant non-confidential results from other related studies that become available. It will be the responsibility of the DMC to determine the extent to which this information is relevant to decisions to continue or modify the current study. The DMC will provide recommendations in writing to the PI to change or stop a study, or part thereof (e.g., one arm), or to continue a study unchanged. Special consideration will be given to participants already in treatment.

In the event that a study change is recommended for participant safety reasons (including early stopping of inferior therapy), the PI acts to implement the change as expeditiously as possible to ensure safety of all participants on the study. In the unlikely situation that the PI does not concur with the DMC recommendation, then the Vice President for Research Administration must be informed of the recommendation of the DMC and of the PI's reason for disagreeing with the recommendation. The Vice President for Research Administration and the PI, in consultation with the DMC Chair, are responsible for

reaching a mutually acceptable decision about the study. Confidentiality is maintained during these discussions. In the event that a change in a study is recommended for reasons other than participant safety (e.g., to extend accrual because of a lower than expected accrual rate), the DMC provides to the PI as much rationale for the proposed change as can be made without jeopardizing the conduct of the study. The PI is responsible for having an amendment prepared and submitted to the IRB the recommendations of the DMC and providing the rationale for the changes. The IND Sponsor – MDACC and IRB approval of the amendment will be required prior to implementation of the change, although a decision to override the DMC's recommendation is made only in the most exceptional circumstances.

All documents, investigative reports or information and conversations relating to this committee's work are strictly confidential and are not shared with anyone other than other committee members. Although committee documents are subject to legal privileges as set forth in statutory and case law and are not subject to discovery during a litigation process, the privilege may be lost if committee documents are given to, shown to or discussed with non-committee members without an official DMC request to do so.

No communication of the deliberations or recommendations of the committee, either written or oral is made outside of the committee except as provided for in these policies and procedures. Statements of confidentiality will be signed by all DMC members or alternates at the beginning of an appointment period. Outcome (efficacy) results are strictly confidential and are not divulged to non-members (excepting the PI and Associate Vice President for Clinical Investigations) until the recommendation to report the results are accepted and implemented.

Individuals invited to serve on the DMC disclose to the Group Chair any potential, real or perceived, conflicts of interest. These include professional interest, proprietary interest and miscellaneous interest considerations. Potential conflicts that develop during the conduct of a trial should also be disclosed to the PI.

Guidelines for Filling Reports of Adverse Experiences at MDACC

Given the non-invasive, minimal risk nature of the proposed research, we anticipate that the types of adverse experiences that may occur, if any, will focus on concerns about medication side effects, phlebotomy, and the discomfort of nicotine withdrawal, possible distress associated and with sensitive issues arising during data collection. We have taken several steps to minimize these risks, as described below.

Adverse Experiences Associated with Nicotine Abstinence/Withdrawal

Participants may experience nicotine abstinence/withdrawal effects. These effects may include irritability, difficulty concentrating, insomnia, anxiety, dysphoria, and increased hunger. None of these effects result in serious adverse health consequences.

Adverse Experiences Associated with Medication Use

Comprehensive screening will be conducted to ensure that all participants with contraindications for either varenicline or bupropion use would get excluded from participation. For bupropion, these conditions include the presence of hypersensitivity to bupropion; use of MAO inhibitors or discontinuation within the past 2 weeks, history of seizures, prior head trauma, recent abrupt discontinuation of alcohol or sedatives (including benzodiazepines), current diagnosis of bulimia or anorexia, recent myocardial infarction, uncontrolled hypertension, tachycardia, hypotension or risk of orthostatic hypotension, unstable heart disease, history/current renal or hepatic disease, use of other psychotropic medications including antidepressants, or drugs that inhibit the CYP2B6 enzyme system, Suicidal ideation and behavior or worsening depression; addiction to opiates, cocaine, or stimulants; diabetes treated with oral hypoglycemics or insulin; excessive alcohol or sedative use insomnia medications or treatments that lower seizure threshold, CNS tumor psychosis and/or mania and use of over-the-counter stimulants and anorectics. For varenicline these conditions include, severe renal impairment and intolerable nausea. In addition, smoking cessation alone may alter the pharmacokinetics or pharmacodynamics of some drugs including theophylline, warfarin and insulin, in which case a dose reduction of such drugs should be considered.

Standard blood chemistries including liver and renal function tests will be ordered on the first screening session and reviewed prior to the subsequent screening visit for acceptability.

The typical side effects are not usually serious in nature and often abate within a few days to weeks after starting medication or once the medication is withdrawn. Adverse effects and concomitant medications will be assessed at each of the post-baseline in-clinic, phone, and counseling sessions. Participants' vital signs, including blood pressure, heart rate, and weight will also be measured at each in-clinic visit. The study physician will monitor participants' complaints of adverse events and, when necessary, adjust the dosage or discontinue medication, order additional lab tests, etc. Adverse experiences and medication assessments will continue until 90 days following completion of the drug regimen.

Adverse Experiences Associated with Blood Collection
Syncope, hematoma, and infection are among the common adverse experiences associated with phlebotomy. To minimize participants' exposure to these adverse effects, trained phlebotomists will be employed to handle all blood collection procedures.

Serious Adverse Event Reporting (SAE)
A serious adverse event is any adverse drug experience occurring at any dose that results in any of the following outcomes:

- Death
- A life-threatening adverse drug experience – any adverse experience that places the patient, in the view of the initial reporter, at immediate risk of death from the adverse experience as it occurred. It does not include an adverse experience that, had it occurred in a more severe form, might have caused death.
- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant disability/incapacity – a substantial disruption of a person's ability to conduct normal life functions.
- A congenital anomaly/birth defect.

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse (21 CFR 312.32).

Important medical events, as defined above, may also be considered serious adverse events. Any important medical event can and should be reported as an SAE if deemed appropriate by the Principal Investigator or the IND Sponsor, Office of Research Education and Regulatory Management (ORERM).

- All events occurring during the conduct of a protocol and meeting the definition of a SAE must be reported to the IRB in accordance with the timeframes and procedures outlined in "University of Texas M. D. Anderson Cancer Center Institutional Review Board Policy on Reporting Serious Adverse Events". Unless stated otherwise in the protocol, all SAEs, expected or unexpected, must be reported to ORERM, regardless of attribution (within 5 working days of knowledge of the event).

- **All life-threatening or fatal events,** expected or unexpected, and regardless of attribution to the study drug, must have a written report submitted within **24 hours** (next working day) of knowledge of the event to the Safety Project Manager in ORERM.

The MDACC "Internal SAE Report Form for Prompt Reporting" will be used for reporting to ORERM.

Serious adverse events will be captured from the time the patient signs consent until 90 days after the last dose of drug. This 90-day time frame may be extended if the PI and/or the study's medical team deem it necessary. Serious adverse events must be followed until clinical recovery is complete and laboratory tests have returned to baseline, progression of the event has stabilized, or there has been acceptable resolution of the event.

Additionally, any serious adverse events that occur after 30-days post treatment that are related to the study treatment must be reported to ORERM. This may include the development of a secondary malignancy.

Reporting to FDA:

- Serious adverse events will be forwarded to FDA by the IND Sponsor (Safety Project Manager ORERM) according to 21 CFR 312.32.

It is the responsibility of the PI and the research team to ensure serious adverse events are reported according to the Code of Federal Regulations, Good Clinical Practices, the protocol guidelines, the sponsor's guidelines, and Institutional Review Board policy.

Reporting to Pfizer Pharmaceutical Company:
Serious adverse events will be reported to Pfizer according to the company's most recent Safety Reference Manual. Currently, Pfizer's Safety Reference Manual requires that all SAEs be reported to Pfizer from the time the subject signs the informed consent document through 28 calendar days after last administration. If the SAE is fatal or life-threatening, it will be reported to Pfizer immediately after the study team becomes aware. All other cases will be reported within 24 hours of knowledge. All SAE's will be reported to Pfizer regardless of attribution (relatedness).

Data Quality and Integrity
Because of the ongoing monitoring of the project, study investigators and staff are responsible for ensuring that data quality assurance procedures are developed and maintained. Several procedures will be used to maintain the integrity of the data. All databases will be stored in a centralized location on one of the departmental servers, which is backed up daily, with access limited to specific users at the discretion of the PI. The PI will assure that audits of selected subsets of data are performed and that appropriate safeguards of participant privacy are maintained. Privacy safeguards will include appropriate password protection and physical security for all computer systems.

Additional quality assurance procedures include a data collection protocol documented in a protocol manual; a two-stage editing procedure for survey data collection consisting of the initial review of the data collection form by a project member immediately following data collection, and a second review by a project member who will record any significant deviations from the protocol; and regular meetings between the study statistician, the PI, data managers, and other project staff to review problems and solutions, and discuss concerns. Data entry systems, whether via a CATI, or QDS, system, scannable forms, or hand entry with verification, specifically provide field checks, range checks for continuous variables and valid value checks for categorical variables; checks for legitimate dates and times and logical consistency. During data collection, we will issue reports weekly, or even following any new data entry, depending on the needs of the project. Queries and reports will be provided to the PI. Preliminary review will be initiated shortly after data collection begins to allow monitoring of data quality.

F. Vertebrae Animals-NA

G. Literature Cited

H. Consortium/Contractual Arrangements - NA

I. Consultants - NA

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