Novel Medication as a Potential Smoking Cessation Aid

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The following is a summary of protocol changes approved by the IRB with the date of approval. The originally submitted and approved protocol follows this summary.

04/06/15	Switched from using the PRIME-MD at the screening visit to using the Patient Health Questionnaire (PHQ)
06/15/15	Added Facebook as a recruitment method
02/26/16	Changed inclusion criteria to lower the required average number of cigarettes smoked per day from 8 to 5 cigarettes

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Study Abstract

Norepinephrine reuptake inhibitors are a class of medications that may be effective at facilitating smoking cessation but are currently understudied. Nortriptyline has been shown to be effective at increasing quit rates in several studies and is thought to work via inhibition of norepinephrine reuptake. Despite its efficacy, nortriptyline use is limited due to its other pharmacological properties (e.g. blockade of muscarinic and histamine receptors) which are responsible for side effects such as dry mouth, constipation, drowsiness and in overdose situations delayed cardiac conduction. Due to its adverse effect profile and lack of FDA indication for smoking cessation, nortriptyline is not currently recommended as a first line therapy for smoking cessation with none of the recommended agents acting primarily via norepinephrine reuptake inhibition. Levomilnacipran is a medication approved for the treatment of depression that inhibits the reuptake of norepinephrine more potently than the reuptake of serotonin. The objective of this study is to provide preliminary information in order to determine if levomilnacipran may be effective at increasing smoking cessation rates. In this cross-over study, generally healthy smokers will be evaluated while receiving levomilnacipran and while receiving placebo. The three week evaluation periods will occur in random order (i.e. one-half of the subjects will receive levomilnacipran followed by placebo, whereas the other half will first receive placebo followed by levomilnacipran). The first week of each three week evaluation period will be to obtain baseline values, the second week to titrate medication and the third week to evaluate efficacy.

1 Purpose and Rationale

Although 70% of smokers indicate a desire to quit, the best current treatments result in less than 30% achieving long-term abstinence.[1] New treatments for tobacco dependence are introduced rarely with only three medications currently approved for increasing smoking cessation rates (i.e. medicinal nicotine, varenicline, bupropion) and one additional medication consistently shown to be effective but rarely used due to concerns about adverse events (i.e., nortriptyline). Clearly, additional therapies are needed.

Norepinephrine reuptake inhibitors are a class of medications that are likely to be effective at facilitating smoking cessation but are currently understudied. Nortriptyline has been shown to be effective at increasing quit rates in several placebo controlled studies and is thought to work via inhibition of norepinephrine reuptake.[2] Despite its efficacy, nortriptyline use is limited due to its other pharmacological properties (e.g. blockade of muscarinic and histamine receptors) which are responsible for side effects such as dry mouth, constipation, drowsiness and in overdose situations delayed cardiac conduction. Due to its adverse effect profile and lack of FDA indication for smoking cessation, nortriptyline is not currently recommended as a first line therapy for smoking cessation.[1] None of the other approved medications act primarily via norepinephrine reuptake inhibition. Therefore, in order to confirm that norepinephrine reuptake inhibition is an important mechanism by which smoking cessation can be facilitated, studies are needed with other norepinephrine reuptake inhibitors that are well tolerated. Levomilnacipran (recently approved for treating major depressive disorder) fits these criteria but there is no data regarding its effect on smoking.

The proposed pilot study will use novel, recently published methods to assess if levomilnacipran is likely to be effective at increasing smoking cessation rates. This rapid assessment study design developed by Perkins et al allows for the efficacy of a smoking cessation medication to be assessed more quickly and at lower cost than if a full scale clinical study were to be conducted.[3-6] Positive results would lead to studies that could more definitively assess the efficacy of this agent at increasing smoking cessation rates. Conversely, negative results would suggest that norepinephrine reuptake inhibition is not an important mechanism by which medications can increase quit rates and that other mechanisms need to be explored.

2 Objectives

Specific Aim and Hypothesis

The central hypothesis of this project is that norepinephrine reuptake inhibitors in general and levomilnacipran in particular are effective at increasing smoking cessation rates.

We are proposing to conduct a within subject, placebo controlled, cross-over study in which 52 subjects will complete a study in which each subject will receive 12 days of placebo and 12 days of levomilnacipran in random order. During the last five days of taking medication or placebo, subjects will attempt to quit smoking with abstinence confirmed at daily visits. The number of days smokers are successfully abstinent will be compared between levomilnacipran and placebo.

The following specific aim will be pursued:

1) Determine the importance of norepinephrine reuptake inhibition as a mechanism by which a medication can increase smoking cessation rates

We hypothesize that levomilnacipran (relative to placebo) will in a laboratory study alter smoking behavior in a manner consistent with a medication effective at increasing smoking cessation rates.

3 Background

The morbidity and mortality associated with cigarette smoking has been well described.[7] The best available therapies, however, result in less than a third of smokers remaining abstinent 6 months after cessation.[1] Clearly, it is imperative that strategies are developed to increase smoking cessation rates. There are currently only two non-nicotine medications that are approved in the United States for smoking cessation. Two additional medications are recommended as second line therapies in clinical practice guidelines (i.e., nortriptyline, clonidine), however only one of these agents (i.e., nortriptyline) has consistent data demonstrating its efficacy, with the recommendation to not use it as first line largely due to its high rates of adverse effects rather than questions regarding efficacy (conversely, the data with clonidine is mixed but meta-analyses have found it to be effective).[1,8] All of the currently available medications therefore have limited efficacy and many have questions regarding their safety (e.g. varenicline has been associated with neuropsychiatric adverse effects; nortriptyline is associated with anticholinergic and antihistaminic side effects and toxicity in overdose). It is critical that new medications are identified to treat tobacco dependence. This is particularly true for smokers of menthol cigarettes (who are predominantly African American) as currently available medications appear to be even less effective in this group.[9]

Nortriptyline efficacy is based on multiple studies in which it was shown to approximately double smoking cessation rates relative to placebo.[2] Although it is an effective antidepressant, its effect on smoking cessation rates are not secondary to the treatment of an underlying depressive disorder since individuals with depression were usually excluded from nortriptyline smoking cessation studies. The pharmacology of nortriptyline is complex, resulting in a variety of both therapeutic and adverse effects. Nortriptyline belongs to the tricyclic antidepressant (TCA) class of medications which inhibit the reuptake of both norepinephrine and serotonin. The ratio of norepinephrine to serotonin reuptake differs considerably among TCAs, with nortriptyline being among the agents that has the greatest degree of norepinephrine reuptake inhibition relative to serotonin reuptake inhibition (the ratio of norepinephrine to serotonin reuptake inhibition being approximately 4:1).[10] Nortriptyline's effects in the treatment of depression, several anxiety disorders and likely in tobacco dependence are due to its ability to inhibit the reuptake of norepinephrine and serotonin. TCAs generally fell out of favor in the treatment of psychiatric disorders due to adverse effects, which is one of the reasons that nortriptyline is currently recommended as a second (rather than a first) line therapy in the treatment of tobacco

dependence. Side effects of TCAs include anticholinergic effects, antihistaminic effects and cardiac conduction delaying effects resulting in side effects such as dry mouth, constipation and drowsiness. Furthermore, in the case of overdose, these drugs can result in potentially serious arrhythmias. Nonetheless, nortriptyline offers a model for a class of medications that may potentially be beneficial in treating tobacco dependence (i.e., norepinephrine reuptake inhibitors).

The proposed study will assess a medication with similar pharmacological therapeutic effects as nortriptyline but without the pharmacological effects thought to be largely responsible for nortriptyline's adverse effects. This medication, levomilnacipran (brand name Fetzima), was approved in 2013 by the Food and Drug Administration to treat major depressive disorder.[11] Like nortriptyline, it inhibits both norepinephrine and serotonin reuptake but with an approximately two fold higher potency at blocking norepinephrine reuptake relative to serotonin reuptake.[12] Unlike nortriptyline, levomilnacipran has no significant affinity for muscarinic or histaminic receptors[11] and therefore lacks many of the side effects that limit nortriptyline's use.

This study is significant in that it will assess if levomilnacipran, a medication already approved for the treatment of major depressive disorder, can potentially also be used to increase smoking cessation success rates. Its pharmacological similarity to nortriptyline suggests that this medication may be effective. Findings suggesting efficacy would likely lead to additional studies to further evaluate levomilnacipran's efficacy in treating tobacco dependence. Identifying a potentially effective new medication to treat tobacco dependence would be highly significant since currently available medications are associated with either problematic side effects and / or poor efficacy.

This study will additionally assess the impact of menthol cigarette smoking on treatment effectiveness. In the United States there are approximately 19.2 million menthol cigarette smokers and the prevalence of menthol smoking is higher among African Americans, women smokers across race/ethnicity and younger smokers. Menthol cigarettes also comprise about 20% of the tobacco industry's sales market and advertising of these types of cigarettes are specifically targeted to women and racial/ethnic minorities. Current medications appear to be less effective in treating menthol smokers leading to sub-optimal treatment options in menthol smoking populations.[9]

4 Study Design

a) General Approach: In order to test if levomilnacipran is a potentially effective aid for smoking cessation, we propose to use methods described by Perkins et al in a series of four recent publications.[3-6] These methods have been demonstrated to be both sensitive and specific for screening novel smoking cessation medications but are less costly and require less time to conduct than a full-scale smoking cessation efficacy study, which usually involve randomizing at least 100 participants into each of two treatment arms (i.e. at least 200 participants total) and following them for a period of over 3 months. Less costly studies have traditionally used laboratory methods to determine if a drug blunts abstinence-induced symptoms such as withdrawal symptoms or if the drug briefly attenuates positive effects of acute smoking by assessing reductions in ad lib cigarette smoking. Unfortunately, these methods are not highly predictive of medication efficacy.[3]

In developing alternative methods, the aim was to be able to combine the practical advantages of laboratory studies with the validity of clinical trials. The result was the development and validation of a within subject cross-over design in which smokers interested in cessation are asked to abstain from smoking for 5 consecutive days. The number of days that smokers can successfully abstain from smoking is compared between a placebo condition and a medication condition. A within subject cross-over design allows a smaller number of subjects to be used

than would be feasible using a parallel design. The sample size needed is further reduced (relative to a smoking cessation clinical study) by using a continuous outcome measure (i.e. number of abstinence days) rather than a quit / not quit dichotomous measure.

In a series of three papers, this method was used to test four medications. Three of the medications tested are known to increase smoking cessation rates (i.e. nicotine patch, bupropion, varenicline) and one medication tested is known to not increase smoking cessation rates (i.e. modafanil). Number of days abstinent (the primary outcome) was significantly higher during use of all three effective medications relative to placebo thereby demonstrating this method's sensitivity.[4-6] The number of days abstinent was not different between modafanil and placebo thereby demonstrating this method's specificity.[5] By examining motivation to quit on outcomes, the developers of this method found that the best results were obtained when those interested in cessation were recruited for studies using these methods.

In order to determine if levomilnacipran is potentially effective at increasing smoking cessation rates, we will use the medication testing methods described and validated by Perkins et al.[3]

b) Study Design Overview: We will utilize a cross-over, double-blind study design. Three weeks of the study will be to evaluate levomilnacipran and three weeks will be to evaluate placebo with the two evaluation periods occurring in random order (i.e. one-half of the subjects will receive levomilnacipran followed by placebo, whereas the other half will first receive placebo followed by levomilnacipran). The first week of each three week evaluation period will be to obtain baseline values, the second week to titrate medication and the third week to evaluate efficacy. In order to determine if effects of levomilnacipran depend on the type of cigarettes smoked (i.e. menthol vs. non-menthol), we will enroll an equal number of those smoking each type of cigarette.

c) Subjects: Volunteers for this study will be recruited through flyers, advertisement and word of mouth. This study will be described in recruitment notices as an evaluation of the short-term effects of a medication to help smokers quit but also 'not as a treatment study.' This is consistent with how similar studies assessing medication efficacy have been advertised.[5] Approximately 19% of the population in Minnesota smokes. There are therefore a sufficient number of smokers in the population to enroll the required number of study subjects. We will be recruiting generally healthy smokers (smoking on average, $\geq$ 8 cigarettes per day) who are motivated to quit smoking. Motivation to quit will be determined by self-report. Due to potential neuropsychiatric side effects of antidepressants in adolescents and young adults, we will only be recruiting subjects over the age of 24. Smokers with medical or psychiatric conditions likely to place them at increased risk if they were to participate in the study or whose conditions might interfere with data interpretation will be excluded. Similarly, smokers taking medication that might interfere with measures to be studied (e.g. psychiatric medications) or that would be expected to interact with or be unsafe to take with levomilnacipran will be excluded.

Inclusion criteria are as follows:

1) Be between $\geq$ 25 years old and $\leq$55 years old
2) Smoke, on average, at least 8 cigarettes per day for a period longer than 1 year
3) Indicate motivation to quit smoking

Exclusion criteria are as follows:

1) Current serious or unstable medical or psychiatric condition (as determined by self-reported medical history, the PRIME-MD and a physical exam)
2) Use of medication that could interfere with measures to be studied (e.g. psychiatric medications) or that would be expected to interact with levomilnacipran (e.g. strong inhibitors of CYP3A4)

3) Substance abuse within six months of beginning the study based on self-report
4) Regularly use any form of nicotine or tobacco other than cigarettes
5) Are pregnant or breast feeding (female subjects will be given a urine pregnancy test at the screening visit with pregnancy later in the study and breast feeding based on self-report)

We are planning to recruit an equal number of menthol and non-menthol cigarette smokers. To qualify as a menthol smoker, greater than 80% of cigarettes smoked are menthol.

c) Procedures

c.1) Screening visit: Initial eligibility will be assessed via a phone interview. Those likely to qualify will be scheduled for a screening visit at which written informed consent will be obtained and eligibility confirmed. Psychiatric exclusion will be based on the Primary Care Evaluation of Mental Disorders (PRIME-MD) instrument. The PRIME-MD, designed to be administered by non mental health professionals, has been found to have good agreement with assessments made independently by mental health professionals.[13] Medical history will be obtained via subject report and a physical exam will be performed to ensure that subjects are in generally good health. A urine pregnancy test will be given to females to confirm that they are not pregnant. Following the screening visit, those subjects who qualify will be scheduled for the remainder of their visits.

c.2) Experimental Protocol: Subjects will participate in two evaluation periods each lasting three weeks. During one of the evaluation periods levomilnacipran will be evaluated whereas during the other evaluation period, placebo will be evaluated. Procedures for the two evaluation periods will be identical, with the order of which period occurs first (i.e. medication or placebo) assigned randomly. The procedures at each study visit are based on published methods. The first week of each period will consist of baseline smoking assessment. This week will also serve as a medication washout period when occurring as the first week of the second three week assessment period (with a terminal half-life of approximately 12 hours,[11] this should be a sufficiently long washout period). During this week subjects will be seen at the research clinic twice. At the second baseline week visit subjects will receive their initial supply of medication. Levomilnacipran will be dosed at 20 mg once daily for two days followed by 40 mg once daily for 10 days. This is consistent with the recommended dose for the treatment of depression.

During the second week of the evaluation period, subjects will begin to take their assigned medication (i.e. levomilnacipran or placebo) and will be seen at two visits. At the first of these visits, subjects will receive a supply of the 40 mg dose of medication (or placebo). Adverse events and subjective measures such as craving and withdrawal symptom severity will be assessed at each visit.

During the third week of the evaluation period, subjects will attempt to abstain from smoking for a five day period and will be seen daily to determine smoking status. These visits will occur on five consecutive days with the visits in most instances occurring Monday through Friday. Subjects will be told to smoke normally until the evening prior to the first of the abstinence assessment days and then to attempt to not smoke at all during the subsequent five days. At each visit, smoking status will be evaluated by measuring exhaled carbon monoxide (CO) concentrations with a value of < 5 ppm confirming abstinence. Each visit during this week will be scheduled in the afternoon in order to assess mid-day CO rather than CO after just waking up. Subject will stop taking medication after they complete their five consecutive days of study visits.

The same procedures will then be repeated except that subjects will receive the alternate treatment. Since continued participation in the study will require subjects to resume smoking, subjects will be told that it is not necessary for them to resume smoking if they wish to remain abstinent; however they would not be able to continue participation in the study if this were the case. At the conclusion of the study, subjects will be given information about resources in the community that could assist them with the smoking cessation attempt. They will additionally be called by telephone approximately 1 week after completing the study in order to ensure that there are no adverse events and to ascertain if they have attempted to quit smoking. Those that have not made a cessation attempt will be encouraged to do so and will again be provided with information regarding community resources to assist them.

Subjects will therefore be seen at a screening visit and at 9 visits per study period for a total of 19 visits.

d) Justification for Drug Dosing: Subject will initiate levomilnacipran at 20 mg daily for 2 days and will then increase the dose to 40 mg once daily for the next 10 days. The recommended dose when used for the treatment of depression is 40 mg to 120 mg. A review of the clinical studies with levomilnacipran however demonstrates that there is little difference in antidepressant efficacy across the dosage range.[14] Furthermore, smoking cessation studies assessing the efficacy of nortriptyline have used doses at the lower end of the recommended dosage range (smoking cessation studies have evaluated daily doses of 75 to 100 mg with the dosage range for depression being 75 mg to 150 mg per day). In order to minimize the risk of adverse events and in order to simplify the titration period (40 mg requires one dosage increase whereas 80 mg per day would require two dosage increases and 120 mg per day would require three dosage increases) we have decided to select a dose at the lower end of the therapeutic range but one that has clearly demonstrated efficacy when used in the treatment of depression. The duration of treatment is based on data demonstrating that bupropion and nortriptyline are successful at increasing smoking cessation rates after one week of treatment. Active medication and placebo will be prepared by the University of Minnesota Investigational Drug Services by over-encapsulating active drug and preparing matching placebo.

d) Subject recruitment and payment: Subjects will be recruited via the use of flyers and advertisements in local newspapers or on internet resources such as Craigslist. Additional recruitment avenues such as radio advertising will be used if necessary. Our research team has considerable experience recruiting subjects into smoking studies and has determined which advertisement strategies are most effective.

Subjects will receive $20 for each visit that they complete with an additional $15 bonus for each day of confirmed abstinence during the abstinence assessment visits occurring at week 3 of each medication period. Subjects who attend all study visits and comply with all study related procedures will receive a $60 bonus (regardless of how successful they are at cessation). Subjects can therefore receive up to $590 for completing the entire study.

e) Outcome Measures: The primary outcome measure is the number of days of confirmed abstinence during the third week of each assessment period (out of a maximum of 5 days). A secondary outcome is the percentage of subjects who are abstinent at all five cessation visits.

Additionally at each visit, subjects will complete the Minnesota Nicotine Withdrawal Scale (MNWS), the Subjective State Scale (SSS) and the Questionnaire of Smoking Urges (QSU-Brief). Subjects will also be asked to report any adverse events they have experienced. The Minnesota Nicotine Withdrawal Scale, is a widely used scale to assess nicotine withdrawal symptoms.[15] The positive affect and distress subscales of the SSS, have been found to be reliable at assessing these mood symptoms (alphas are 0.85 and 0.82, respectively).[16] The Brief version of the Questionnaire of Smoking Urges (QSU-Brief) is a commonly used scale to assess nicotine craving with a score for each of two factors (i.e. intention and desire to smoke,

anticipation of relief from negative affect) calculated in order to examine several aspects of the urge to smoke.[17]

5 Potential Risks of Study Participation and Protection Against Risk

a. Risks to subjects: Levomilnacipran is generally well tolerated with the most common side effects reported being nausea / vomiting, constipation, excessive sweating, increased heart rate, urinary hesitation, palpitations and erectile dysfunction. Although occurring rarely other side effects including but not limited to mania, rash and allergic reaction, mydriasis, increases in blood pressure and abnormal bleeding have been occasionally reported. There is the possibility that when discontinuing levomilnacipran, subjects may experience a withdrawal syndrome that includes symptoms of dizziness, nausea and paresthesias. Questionnaires will be used to assess a variety of measures to ensure eligibility for the study and to measure outcomes of interest such as cigarette craving and withdrawal symptoms. Some of these questionnaires may ask questions that subjects would consider personal.

As with any medication, there is a risk that unanticipated side effects may occur.

b. Adequacy of protection against risk: This study will be submitted for approval by the University of Minnesota Institutional Review Board. The study will be explained to subjects by an investigator or a study coordinator trained in the protection of human subjects (per university guidelines) and subjects will have an opportunity to ask any questions prior to signing an informed consent form.

Levomilnacipran, like all antidepressants, has a warning regarding increased risk of suicidal thinking and behavior in children, adolescents and young adults. For this reason, we are limiting enrollment to those over the age of 24. Additionally, we are limiting enrollment to generally healthy individuals ages 55 and under. Individuals with medical or psychiatric conditions likely to place them at increased risk of being in the study will be excluded. Medical and psychiatric history will be obtained via subject report and a physical exam will be performed to ensure that subjects are in generally good health. Similarly, smokers taking medication that might interfere with measures to be studied (e.g. psychiatric medications) or that would be expected to interact with or be unsafe to take with levomilnacipran (e.g. strong CYP3A4 inhibitors) will be excluded. Additionally, we are using a relatively low dose of medication (the dose being used is the lowest recommended dose when used for the treatment of depression). Although many side effects are not reported to be dose related, side effects such as erectile dysfunction and urinary hesitancy appear to be.[11] The risk of these side effects should be decreased due to the low dose being used as should the risk of withdrawal symptoms after discontinuation of the medication.

Subjects will be informed that they are free to not answer any questions that they are uncomfortable with, however by doing so they may be excluded from the study if eligibility cannot be assessed or dropped from the study if measures of interest cannot be ascertained.

To ensure confidentiality, all subjects enrolled in the study will be assigned a study identification code. These codes will be used on all study related data collection forms except for those on which the use of personal identifiers is mandatory (e.g. informed consent form). Forms that link the name of the participant and the subject identification code will be kept in a locked cabinet or office or in an electronic file stored on password protected computer servers meeting University guidelines. Access to subject identifiable information will be limited to those that require this information such as study investigators or others who have direct contact with study subjects (e.g. study coordinator).

6 Statistical Analysis

a. Statistical Methods of Data Analysis

a.1) Statistical Design: A within-subject cross-over design will be used for the study in which smokers will receive levomilnacipran and placebo (double-blind) with the order of treatment being randomly assigned. The primary aim is to compare the number of abstinent days between levomilnacipran and placebo by type of cigarette smoked (menthol cigarette and non-menthol cigarette). A secondary aim is to compare levomilnacipran responses between menthol cigarette smokers and non-menthol cigarette smokers.

a.2) Outcome Variables and Measurements: The primary outcome is the number of days abstinent during the third week of each assessment period as assessed by self-report of no smoking that day and verified by an exhaled carbon monoxide (CO) concentration of <5 parts per million. A secondary outcome is the percentage of subjects who are abstinent at all five cessation visits. The other outcomes include ratings on the Minnesota Nicotine Withdrawal Scale (MNWS), the Subjective State Scale (SSS), the Questionnaire of Smoking Urges (QSU-Brief) and frequency of reported adverse events.

a.3) Hypotheses: The following hypothesis will be tested: 1) Levomilnacipran increases the number of abstinent days vs. placebo. This hypothesis will then be tested separately for smokers of menthol cigarettes and for smokers of non-menthol cigarettes. 2) A secondary hypothesis is that the increase in number of abstinent days will be lower in smokers of menthol cigarettes than in smokers of non-menthol cigarettes (i.e. levomilnacipran will be less effective in smokers of menthol vs. non-menthol cigarettes).

a.4) Data analysis: A paired t-test will be used to test treatment effect based on days of abstinence per quit assessment week (range 0-5, not necessarily consecutive) between levomilnacipran and placebo. Additional separate analyses for menthol and non-menthol cigarette smokers will be conducted. A two-sample t-test will be used to compare the difference in days of abstinence per quit assessment week for menthol cigarette smokers compared to non-menthol cigarette smokers.

Repeated measures Analysis of Variance (ANOVA) will be used for days of abstinence per quit assessment week, assuming mixed effects three-factor ANOVA model (“Order of phases” with two levels, “Treatment” with two levels, “Menthol cigarettes smoker” with two levels, subject effects are random); then use contrasts to compare treatment with placebo for all participants and for menthol cigarette smokers and non-menthol cigarette smokers separately. This approach allows us to investigate primary and secondary aims simultaneously, therefore, to potentially achieve higher statistical power. The use of paired t-tests, on the other hand, provides a proper tool for sample size determination as seen in the following section.

Generalized estimating equation (GEE) model will be used to analyze the longitudinal data, with the number of subjects able to maintain continuous abstinence on all 5 days throughout the quit week as the secondary outcome. Treatment effect (levomilnacipran vs. placebo) and cigarette type effect (menthol vs. non-menthol) will be estimated.

Secondary variables such as scores on the MNWS, SSS and QSU-Brief will be summarized by the mean, standard deviation, median and range. The change in these measures occurring between baseline (i.e. the first week of treatment) and the last day of the third week will be compared between drug and placebo. Similar two-factor ANOVAs will be used to analyze each of the calculated differences as the primary endpoint. Additionally, frequency of subject reported adverse events will be compared between treatments via the use of paired t-tests.

a.5) Power Consideration: The adequacy of planned sample sizes are assessed using two-sided paired t-test. We tested the “minimum detectable difference”, expressed as the number of standard deviations (i.e. “effect size”) for several sample sizes where the two-sided paired t-test is pre-set at a 5% significance level and 80% power. The variability is based on estimates found

in the literature by others using these methods.[5] With a total of 52 subjects completing the study (26 menthol smokers and 26 non-menthol smokers), we are able to detect an effect size of 0.57 standard deviations for the comparisons between levomilnacipran and placebo which is considerably smaller than the effect size of approximately 1.65 reported by Perkins et al for bupropion.[5] Since we anticipate a 20% attrition rate, we are planning on enrolling 64 smokers in total (32 smokers of menthol cigarettes and 32 smokers of non-menthol cigarettes).

7 Data and Safety Monitoring Plan (DSMP)

The principal investigator in conjunction with the study physician will be responsible for monitoring adverse effects. At the conclusion of each visit subjects will be asked whether they have had any side effects. Any positive response will be followed up with questions regarding details of what the adverse effect was and the severity. To the extent possible, each adverse event will be evaluated to determine 1) its severity (mild, moderate, severe), 2) its relationship to the study medication; 3) its duration and 4) whether it constitutes a serious adverse event.

The participation of any subject will be discontinues if they develop a side effects of greater than moderate severity (as determined by the investigators) or develops any symptoms that in the opinion of the investigators would warrant discontinuation from the study. Participation in the study would also be discontinued if during the course of the study the subject develops a significant medical condition (regardless of whether it can be attributed to the study drug) or is initiated on medication that can be expected to interact with the measures being assessed or increase the risk of being on the study medication. The subject has the option of discontinuing their participation at any time for any reason. Adverse events will be reported to the IRB as required per IRB guidelines.

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