

EVALUATION METHODS

Measurement of abstinence. At each clinic visit, subjects will complete items pertaining to their smoking status. Specifically, we will query them as to their *Smoking Status at the Time of the clinic visit; Smoking Status Since the Previous Clinic Visit and Smoking Status Since Quitting*. This will enable us to measure point prevalence abstinence, prolonged abstinence and continuous abstinence (see below). In addition, we will measure expired-air carbon monoxide at each clinic visit (including all follow-up visits).

Prolonged Abstinence. PA will be defined as a report of non-smoking following an initial 2-week grace period during which any smoking is not counted as a failure. Here, failure is defined as either seven consecutive days of smoking or smoking on at least one day on each of two consecutive weeks.

Point Prevalence Abstinence. PP will be defined as no smoking, (not even a puff), for 7 consecutive days prior to the clinic visit and an expired-air CO level of <9PPM.

Primary analysis of treatment effects. We will model the primary outcomes of this study in two ways. One primary analysis will focus on abstinence. All those who fail to attend follow-ups and provide biochemical confirmation of abstinence will be considered as treatment failures. We will also examine the effects of treatment on relapse which entails a less conservative definition of treatment effects. Finally, we will employ random regression models to examine secondary research questions. Since missing data is a known plague of treatment outcome research, the use of random regression models provides an attractive way of coping with missing data. These approaches are described in more detail below.

Primary Analysis of effects of maintenance treatment on abstinence. Logistic regression analysis will be conducted to examine differences in abstinence between those receiving the experimental and control maintenance treatments. Separate models will be fitted for each follow-up point using biochemically-confirmed smoking status as the dependent variable and treatment condition as the independent variable.

Expired Air Carbon Monoxide. We will verify self-reports visits by conducting expired-air carbon monoxide analysis (cotinine cannot be used during NRT). Expired air CO provides a useful index of tobacco exposure.

EXAMINATION OF TREATMENT EFFECTS ON LATE RELAPSE VIA SURVIVAL ANALYSIS

In addition to the analysis of treatment effects based upon prolonged abstinence, we will use survival analysis to explore the effects of treatment condition and several baseline variables on late relapse.

Definition of relapse. Relapse will serve as the dependent variable for the multivariate survival analysis. In keeping with the recommendations of the National Working Conference on Smoking Relapse, *a relapse will be defined as at least seven consecutive days of smoking* (Ossip-Klein et al, 1986). The relapse date will be treated as the first day on which smoking occurs for seven consecutive days.

Alternative definitions of relapse. We will also include the following definition suggested by Ockene et al. Relapse: smoking five or more cigarettes per day for 3 consecutive days; Lapse: taking even a puff. We will conduct secondary analyses as described below using both this definition of relapse and the definition offered by Ossip-Klein et al.

SECONDARY ANALYSES

We view the secondary analyses as falling into the categories of risk assessment and targeting studies. The following measures will be obtained for inclusion in these studies. We then provide a description of the targeting studies that we plan to conduct.

Vital Signs. Heart rate and blood pressure will be measured with an automated blood pressure device (DINAMAP XL 9300, Johnson & Johnson Medical, Inc.) throughout acute and relapse prevention phases of the study.

Modified Fagerstrom Test for Nicotine Dependence. This instrument consists of five questions modified from the Fagerstrom Tolerance Questionnaire (Fagerstrom, 1978). Test-retest reliability data collected over the telephone for each of the questions has been previously established (Killen et al, 1990).

Withdrawal Symptoms. DSM-IV derived ratings of withdrawal, as described by Hughes and Hatsukami (1986) will be assessed throughout the study.

Craving. We will use the Questionnaire of Smoking Urges (QSU) developed by Tiffany and colleagues in order to examine the effects of treatment on craving associated with the memory of positive reinforcement as well as craving associated with the anticipation of relief of withdrawal symptoms. The QSU is a 32 item instrument developed by Tiffany & Drobes (1991) to tap four conceptually distinct aspects of smoking urges: desire to smoke, anticipation of positive outcomes from smoking; anticipation of relief from abstinence effects associated with nicotine withdrawal and intention to smoke. We will also use the measure of craving we have developed in previous work (Killen et. al, 1991; 1992; 1997).

The Structured Clinical Interview for DSM-IV (SCID). The SCID (mood disorders portion) will be administered to all participants at study entry (baseline). The SCID is a semi-structured interview for making Axis I DSM-IV diagnoses.

Center for Epidemiological Studies Depression Scale (CES-D). The CES-D will be used to measure level of depression symptoms at baseline and throughout the acute and maintenance phases of treatment (Radloff, 1977). The CES-D is a short self-report scale designed to measure depressive symptomatology in the general population. The items of the scale are symptoms associated with depression in previously validated scales.

Body Mass Index. BMI will be computed from the formula kg/m^2 . Height and weight will be recorded on a standard balance beam scale. Participants will remove shoes, jackets and any additional heavy clothing before they are measured. We include a measurement of body weight given interesting data we have recently reported suggesting interactions with nicotine dependence and depression (Killen et al, 1996). We wish to confirm these observations in the proposed study. Height and weight will be assessed at all clinic visits.

Genetic data

Allele frequencies for polymorphisms to be used as predictors of treatment outcome. We have developed genotyping assays for a number of the polymorphisms that we propose to test, and validated the presence of these variants in test samples of DNA. The polymorphisms we propose to study are as follows.

Nicotinic Acetylcholine Receptors

Alpha 4 subunit (1) rs1044393 – exon synonymous C = 87.5%; (2) rs755203 – promoter C = 36%

Plus one other variant to be identified

Alpha 6 subunit (1) rs892413 – intron G = 70%; (2) rs2304297 – 3' regulatory Plus one other variant to be identified; *Beta 2 subunit* (1) rs2072660 – exon UTR A = 28%; (2) G2053T – intron G = 90%; *Beta 3 subunit* Three variants to be identified.

Dopamine Metabolism, Synthesis, Reuptake and Action

COMT: (1) rs165688 – exon nonsynonymous A = 50% allele (2) other variants to be identified

TH: Three SNPs chosen from the 9 that are listed in public databases in regulatory, exon, exon-intron boundary regions.

DAT(SLC6A3): (1) 3' tandem repeat (Sabol et al 1999) 9 repeat allele 24.8%; 10 repeat allele 75.2%

Two regulatory region variants to be identified – little variation is found in coding region of this gene.

DRD2 A1/A2 Taq alleles: (3' UTR – rs1800497) A2 = 72% allele frequency; B1/B2 Taq alleles (intron – rs1079597) one other SNP from 14 additional exon, 5' and 3' variants identified by SHGC.

MAO-A: (1) rs6323 (exon nonsynonymous) T = 54%; (2) rs1801291 (exon nonsynonymous); (3) rs979606 (exon-intron boundary)

MAO-B: (1) 3' UTR (C116114T) – identified by SHGC T = 80% ; (2) promoter – identified by SHGC (3) intron – identified by SHGC G = 67%

Norepinephrine Synthesis and Reuptake

DBH (1): C-1021T promoter variant – assay under development; (2) G444A – assay under development; One other variants to be determined

NET (1): rs2242446 – promoter C = 69%; (2) rs – exon synonymous G = 60%;

Risk Assessment and Targeting Studies will include:

1. Effects of treatment in reducing the severity and frequency of depression symptoms and MDD. The CES-D will serve as our dependent measure of depression symptoms for this analysis. Random Regression Models (RRM) will be used to compare the efficacy of the two treatment conditions in reducing the severity and frequency of depression symptoms as measured by the CES-D..

2. Effects of treatment in reducing the severity and frequency of craving and DSM-IV withdrawal symptoms. RRM's will be used to compare the efficacy of the two conditions in reducing the severity and frequency of craving and other withdrawal symptoms associated with nicotine deprivation.

3. For pharmacogenetic analysis, clinical outcome measures will be used as dependent variables, and genotype (3 levels or 2 levels, depending on genotype frequencies) will be used as predictors. For discontinuations due to adverse events, Kaplan-Meier survival analyses will be used. Potentially important covariates such as age, baseline body weight, and baseline cigarette use will be included in analyses. Type I error is a major concern given the large number of predictor SNPs and multiple outcome measures. We will report unadjusted p-values and p-values adjusted for multiple comparisons. Unadjusted p-values reflect the expected rate of false positives among all tests and can be converted into estimates of the numbers of true and false positive results. We will use multilocus permutation procedures to adjust p-values for multiple comparisons to reflect the family-wise error rate or probability that any of the tests results in a false positive result (Lazzeroni and Lange 1998). Such procedures can be far more powerful than standard Bonferroni corrections when the tests are positively correlated as is expected for related outcome measures and for SNPs that are in linkage disequilibrium with each other. Power is also a concern. In our sample, for a SNP with a minor allele frequency of .20, an overall response rate of 46%, and a response rate of 70% in carriers and 30% in noncarriers, the power to predict is close to 1.0 using an alpha of 0.005 (McCarthy and Hilfiker 2000). Data will be analyzed with and without minorities included.