Published in final edited form as:

Lancet Respir Med. 2015 February ; 3(2): 131–138. doi:10.1016/S2213-2600(14)70294-2.

A Randomized Placebo-controlled Trial to Test a Genetically-informed Biomarker For Personalizing Treatment for Tobacco Dependence

Caryn Lerman, Ph.D.¹, Robert A. Schnoll, Ph.D.², Larry W. Hawk Jr., Ph.D.³, Paul Cinciripini, Ph.D.⁴, Tony P. George, M.D.⁵, E. Paul Wileyto, Ph.D.⁶, Gary E. Swan, Ph.D.⁷, Neal I. Benowitz, M.D.⁸, Daniel F. Heitjan, Ph.D.⁶, Rachel F. Tyndale, Ph.D.^{5,9}, and on behalf of the PGRN-PNAT Research Group*

¹Department of Psychiatry, Annenberg School for Communication, and Abramson Cancer Center, University of Pennsylvania, 3535 Market Street, Suite 4100, Philadelphia, PA, 19104

²Department of Psychiatry and Abramson Cancer Center, University of Pennsylvania, 3535 Market Street, Suite 4100, Philadelphia, PA, 19104

³Department of Psychology, University at Buffalo, SUNY, Buffalo, NY, 14260

⁴Department of Behavioral Science, The University of Texas MD Anderson Cancer Center, Cancer Prevention Building (Room CPB3.3309) 1155 Pressler, Houston, TX, 77030

⁵Centre for Addiction and Mental Health(CAMH) and Department of Psychiatry, University of Toronto, 1001 Queen St. West, Toronto, Ontario, Canada M6J 1H4

Corresponding Author: Caryn Lerman, Ph.D., Mary W. Calkins Professor, Department of Psychiatry, University of Pennsylvania, 3535 Market Street, Suite 4100, Philadelphia, PA 19104; Phone: (215) 746-7141; Fax: (215) 746-7140; clerman@upenn.edu.

*PGRN-PNAT Research Group: Pharmacogenomics Research Network: Pharmacogenetics of Nicotine Addiction Treatment Research Group (Frank Leone, M.D. claims authorship responsibility on behalf of this group). Includes: Frank Leone, M.D.; Henry Glick, Ph.D.; Angela Pinto, MBA; Paul Sanborn, M.S.; Elissa Kranzler, M.S.Ed.; Peter Gariti, Ph.D.; Richard Landis, Ph.D. (University of Pennsylvania); Maria Novalen, M.Sc.; Adel Aziziye, M.Sc. (CAMH/University of Toronto); Martin Mahoney, M.D., Ph.D. (Roswell Cancer Center, University of Buffalo); Maher Karam-Hage, M.D. (The University of Texas M.D. Anderson Cancer Center); David Conti, Ph.D. (University of Southern California); Andrew Bergen Ph.D. (SRI International).

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Declaration of Interest

Lerman received study medication and placebo, as well as support for medication packaging, from Pfizer. She has also consulted to Gilead, and has been a paid expert witness in litigation against tobacco companies. Cinciripini served on the scientific advisory board of Pfizer Pharmaceuticals, conducted educational talks sponsored by Pfizer on smoking cessation from 2006-2008, and has received grant support from Pfizer. Schnoll received medication and placebo free of charge from Pfizer for a different project, and has consulted to Pfizer and GlaxoSmithKline. George has had both investigator-initiated and industry-sponsored grants from Pfizer in the past 12 months, and serves on a Data Monitoring Committee for Novartis. Benowitz has served as a consultant to several pharmaceutical companies that market smoking cessation medications and has been a paid expert witness in litigation against tobacco companies. Tyndale has acted as a consultant to pharmaceutical companies, primarily on smoking cessation. The remaining authors report no conflicts of interest.

Contributors

CL and RT conceived of the study and obtained funding. RS, EPW, NB, GS, and DH assisted with conceiving of the study and preparation of the application for funding. RS, LH, PC, and TG supervised data collection at the 4 sites. EPW and DH supervised data analysis. Members of the PGRN-PNAT Research Group were involved in data collection and study implementation. All authors contributed to the writing of this manuscript. CL is guarantor of this article and all authors have read and approved of the manuscript.

⁶Department of Biostatistics & Epidemiology, University of Pennsylvania, 619 Blockley Hall 423 Guardian Drive, Philadelphia, PA 19104

⁷Department of Medicine, Stanford University, Palo Alto, CA, 94305

⁸Departments of Medicine, and Bioengineering & Therapeutic Sciences, University of California, San Francisco, San Francisco, CA, 94143

⁹Department of Pharmacology & Toxicology, 1 King's College Circle, University of Toronto, Toronto, Ontario, Canada, M5S 1A6

Summary

Background—There is substantial variability in therapeutic response and adverse effects with pharmacotherapies for tobacco dependence. Biomarkers to optimize treatment choice for individual smokers may improve treatment outcomes. We tested whether a genetically-informed biomarker of nicotine clearance, the nicotine metabolite ratio (NMR; 3'-hydroxycotinine/cotinine), predicts response to nicotine patch vs. varenicline for smoking cessation.

Methods—An NMR-stratified multicenter, randomized, placebo-controlled clinical trial was conducted from November 2010–September 2013 at 4 sites. Treatment-seeking smokers (1246: 662 slow metabolizers; 584 normal metabolizers) were randomized to 11-weeks of nicotine patch (active patch + placebo pill), varenicline (active pill + placebo patch), or placebo (placebo pill + patch), plus behavioral counseling; an intent-to-treat analysis was conducted. Participants were followed for 12-months following the target quit date. The primary endpoint was biochemically verified 7-day point prevalence abstinence at the end of treatment (EOT) to estimate the pharmacologic effect of treatment by NMR. Secondary outcomes were side-effects, withdrawal symptoms, and 6- and 12-month abstinence rates. ClinicalTrials.gov registration: NCT01314001

Findings—In the longitudinal model including all time points, the NMR-by-treatment interaction was significant (ratio of odds ratios (ORR)=1.96; CI=(1.11, 3.46); p=0.02). The results indicate that varenicline was more efficacious than nicotine patch for normal metabolizers, while the efficacy was equivalent for slow metabolizers. In cross-sectional analyses, the interaction was significant at EOT (ORR)=1.89; CI=(1.02, 3.45); p=0.04 and at 6-months (ORR)=2.07; CI=(1.01, 4.22); p=0.05, but not at 12-months (p=0.14). An NMR-by-treatment interaction showed that slow (vs. normal) metabolizers reported greater overall side-effects severity with varenicline vs. placebo (β =1.06; CI=(-2.08, -0.03); p=0.044).

Interpretation—Treating normal metabolizers with varenicline and slow metabolizers with nicotine patch may optimize quit rates while minimizing side-effects.

Funding—National Institutes of Health

Introduction

Despite substantial reductions in tobacco use since the 1960s, rates of tobacco use have remained stable in the U.S. for the past decade¹ and have increased in low-income countries.² Worldwide, about 6 million annual deaths are attributable to tobacco use,² and \$200 billion is spent on tobacco-related healthcare costs.³ FDA-approved smoking cessation treatments include nicotine replacement therapies (NRTs), bupropion, and varenicline.

Although transdermal nicotine patch is the safest and most widely used form of pharmacotherapy in the US and Europe,⁴ end-of-treatment quit rates in clinical trials rarely exceed 30%.⁵ The efficacy of nicotine patch is comparable to bupropion,⁶ but may be lower than varenicline.^{7, 8} However, varenicline's efficacy may be offset by the greater likelihood of side-effects.⁹ The substantial individual variability in therapeutic response and side-effects provides a strong rationale to validate novel biomarkers to optimize pharmacotherapy choice.¹⁰

We identified a genetically-informed biomarker of nicotine clearance, specifically the ratio of two metabolites derived from nicotine during smoking, 3'-hydroxycotinine/cotinine, referred to as the nicotine metabolite ratio (NMR). The NMR reflects the activity of the liver enzyme CYP2A6, the major nicotine- and cotinine-metabolizing enzyme. A significant advantage of the NMR over CYP2A6 genotyping is that it incorporates both genetic and environmental (e.g., estrogen) influences on CYP2A6 activity and nicotine clearance.¹¹ Retrospective analyses of prior randomized trials have shown that slow metabolizers (SMs) (lower NMR values and rates of nicotine clearance) respond well to nicotine patch, with no incremental benefit from the non-NRT medication bupropion; normal metabolizers (NMs) do more poorly than SMs on nicotine patch, but benefit from bupropion.¹²⁻¹⁵ To date, no study has examined whether the NMR predicts the efficacy of varenicline, a widely used non-NRT medication that is more efficacious than bupropion.^{16, 17}

To translate these findings to practice, we conducted the first NMR-stratified placebo-controlled randomized trial of nicotine patch vs. varenicline among 1,246 smokers. Although CYP2A6 does not contribute to varenicline metabolism, prior bupropion trial data¹⁴ suggested that a non-NRT medication would aid quitting among NMs. Among NMs, we expected varenicline to be more efficacious than nicotine patch, while among SMs, we expected nicotine patch and varenicline to be equally efficacious.

Methods

Design and Participants

Participants were randomized, by NMR group, to one of three treatment arms: (1) nicotine patch (placebo pill + active patch); (2) varenicline (active pill + placebo patch); or (3) placebo (placebo pill + placebo patch) (Figure 1). Our primary aim was to contrast the efficacy of nicotine patch vs. varenicline by NMR group (NMs vs. SMs). A placebo condition was included to examine side-effects of treatment by NMR group. The primary endpoint was point prevalence abstinence at the end of treatment (EOT) to estimate the pharmacologic effect by NMR group during the medication period. Six- and 12-month follow-up data are presented as secondary endpoints.

We conducted the clinical trial at four academic medical centers (University of Pennsylvania (UPenn), Centre for Addiction and Mental Health/University of Toronto, State University of New York at Buffalo, and MD Anderson Cancer Center); assessment of the NMR was performed at the University of Toronto. From November 2010–September 2013, we recruited participants through advertisements for a free smoking cessation program. Eligible

participants were 18–65 years old and reported smoking 10 cigarettes/day for 6 months (verified by carbon monoxide (CO) >10 ppm).

Exclusion criteria included: 1) use of non-cigarette tobacco products, e-cigarettes, or current smoking treatment; 2) history of substance abuse treatment, current use of cocaine or methamphetamine, or >25 alcoholic drinks/week; 3) medical contraindications (pregnancy, history of cancer, kidney or liver disease or transplant, clinically significant cardiac dysrhythmias, stroke, angina, heart attack, or uncontrolled hypertension); 4) history of DSM-IV Axis I psychiatric disorder or suicide risk score on the MINI International Neuropsychiatric Interview (MINI) >1, or current major depression; 5) current use of antipsychotics, stimulants, opiate medications, anti-coagulants, rescue inhalers, antiarrhythmics, or medications altering CYP2A6 activity (e.g., monoamine oxidase inhibitors, tricyclic antidepressants); and 6) inability to provide informed consent or any condition that could compromise safety.

Randomization and Masking

A biostatistician, independent of the study investigators, developed the randomization procedure which was integrated into a centralized data management system. Subjects were randomized to the treatment arms in a 1:1:1 ratio. Randomization was stratified by baseline NMR status and study site, and blocked in blocks of 12 patients (4/treatment/block) to ensure approximate balance. Participants, study investigators, and personnel (except for the biostatistician and senior data manager) were masked to treatment arm allocation and NMR status. Data were unmasked for analysis following collection of all 6-month follow-up data.

Procedures

The institutional review boards at all sites approved the protocol. Participants eligible at telephone screening completed an in-person medical exam and psychiatric history, completed self-report measures of demographics and smoking history, and provided blood samples for the NMR assay. NMR results, reported within 7 days, were used for final eligibility determination. SMs were oversampled to create a study sample comprising roughly equal numbers of SMs and NMs. For the first 25% of participants enrolled, the NMR cutoff for stratification (and oversampling of SMs) was 0.26 based on a prior clinical trial of NRT.¹⁵ However, to ensure achievement of recruitment goals, the Data and Safety Monitoring Board made a determination (masked to study outcomes) to increase the stratification cut-off to 0.31 (redefining SMs as NMR < 0.31 and NMs (including rapid metabolizers) as those with NMR > 0.31). This cut-off was based on graphical representation of the NMR in the screened population (Supplemental Figure 1A), and was used in all analyses.

Following an in-person 1-hour pre-quit group counseling session at the local clinical site (week-1), varenicline (or matching placebo) was initiated using the guidelines for one week precessation dose titration. Nicotine patch (or matching placebo) was initiated on the morning of the target quit date (TQD). Brief (~15 minute), protocol-driven, telephone counseling was delivered by two counselors at UPenn (weeks 0 (TQD), 1, 4, 8). Counseling focused on skills to quit and avoid relapse, instructions on the use of medication, and

medication compliance. Staff at UPenn collected mid-treatment data (e.g., withdrawal, side-effects) by telephone. Self-reported smoking status was assessed using a standard timeline follow-back procedure,¹⁸ and biochemically verified (see below).

The UPenn Investigational Drug Service distributed medication to the clinical sites. Active patches were purchased from GlaxoSmithKline (Nicoderm CQ®) and identical placebo patches were purchased from Rejuvenation Labs (Salt Lake City, UT). Participants received 11 weeks of patches to match the duration of varenicline following the TQD: 21 mg (6 weeks), 14 mg (2 weeks), and 7 mg (3 weeks). Pfizer manufactured active varenicline and matching placebo pills. Varenicline was delivered for 12 weeks (1 week pre-TQD) as in past trials:¹⁷ Days 1–3 (0.5mg once daily); Days 4–7 (0.5mg twice daily); and Days 8–84 (1.0mg twice daily).

Measures

We assessed demographics, smoking rate, and nicotine dependence (Fagerström Test for Nicotine Dependence).¹⁹ The MINI determined lifetime history of Axis I psychiatric diagnoses. For the NMR assay, cotinine and 3'-hydroxycotinine were assessed by LC-MS; limits of quantification were <1 ng/ml whole blood.²⁰

The primary endpoint was 7-day point prevalence abstinence at EOT (week 11). This outcome was chosen based on guidelines for smoking cessation trials.²¹ Abstinence was defined as no self-reported smoking (not even a puff) for at least 7 days prior to the telephone assessment, with in-person verification for those self-reporting abstinence (CO < 8ppm).²¹ As is the convention, participants lost to follow-up were considered smokers.²¹ To estimate the pharmacologic effect by NMR, subjects should be on medication; therefore, the EOT quit rate was the primary end point. Six- and 12-month quit rates were secondary endpoints to assess whether pharmacogenetic effects persisted after treatment was discontinued.

Self-reported withdrawal symptoms were measured using the Minnesota Nicotine Withdrawal Scale (pre-quit and weeks 0, 1, 4).²² A self-report checklist measured the severity of common side-effects (none [0] to severe [3]), at weeks 0 (TQD), 1, and 4, and was summed to create a side-effects index (we refer to these as “side-effects”, rather than adverse events to distinguish these responses from the open-ended serious adverse events (SAEs) reported by participants).¹⁵ We assessed self-reported use of pills and patches at each visit and collected unused patches and pill blister-packages to confirm self-reports.²³

Statistical Analysis

To test the primary hypothesis of an NMR-by-treatment interaction at EOT, we estimated a logistic regression model using all data. This model included parameters for the odds ratios (ORs) for the treatment effects (nicotine patch vs. varenicline) within SMs and NMs. One measures the interaction effect as the ratio of odds ratios (ORR), calculated by exponentiating the coefficients corresponding to the interaction terms in the logistic model. These models were repeated for 6- and 12-month cessation outcomes. We also used longitudinal logistic regression (General Estimating Equations, GEE) to examine the NMR-by-treatment interaction incorporating all time points. All models controlled for study site;

we also tested our multivariate models controlling for sex, cigarettes per day, nicotine dependence, and race. Self-reported side-effects (continuous measure from the checklist) were examined for each active treatment (vs. placebo) within each NMR group using GEE models including time-point, and adjusted for study site and pre-quit levels of side-effects; the NMR-by-treatment interaction was estimated as a difference of differences (beta coefficients, which estimate mean differences in side effect severity).²⁴ The correlation structure used in the GEE model for side effects utilized subject specific random effects. The model was linear and the measures were continuous. Assumptions were assessed and the distribution was non-normal. However, we verified that we obtained the same results using bootstrap methods (with exact confidence intervals) that are not sensitive to violations of normality. Lastly, we conducted receiver operating curve (ROC) analyses with abstinence as the binary response and NMR as the continuous predictor, separately in the three treatment arms. We tested for heterogeneity among ROC values using the methods of DeLong and colleagues²⁵ as implemented in Stata ROCCOMP.

Our original target sample was 1350. The Data Safety and Monitoring Board conducted a masked interim futility analysis, based on the conditional power method,²⁶ in Feb 2013. In addition to this analysis, we recalculated the power and determined that an N of 1200 would provide adequate power. Specifically, the sample size of 1,200 provided 80% power to detect an ORR of 3.2 for the NMR-by-treatment (nicotine patch vs. varenicline) interaction at EOT. In models that included the placebo arm, the full ITT sample of 1246 participants (662 SM; 584 NM) was used. In models that compared varenicline to nicotine patch, the ITT sample of 838 was used. Stata (Version 13; Stata Corporation; College Station, TX) was used for the analyses.

Role of Funding Source

No funder had a role in study design, collection and analysis of data, writing of the manuscript, or in the decision to submit this manuscript for review. The corresponding author had complete access to all data.

Results

Sample Characteristics

Retention rates at EOT exceeded 70% and retention did not vary across treatment arms or NMR group (Figure 1). The treatment arms did not differ on demographic and smoking history variables (Table 1). SMs were younger ($p=0.02$), less likely to be Caucasian ($p<0.0001$), more likely to be male ($p=0.001$), and smoked fewer cigarettes/day ($p<0.001$), vs. NMs, as reported previously.²⁷ Sex differences in the NMR groups are expected due to estrogen effects on nicotine metabolism rate,²⁷ and the ethnicity difference is expected due to differences in the frequency of reduced/inactive *CYP2A6* alleles. The average NMR for SMs was 0.20 (SD=0.07) and for NMs was 0.50 (SD=0.18). Supplemental Figure 1 shows the distribution of NMR in the screened population ($n=1733$) and in the ITT sample ($n=1246$) where SMs were oversampled.

Abstinence(Figure 2)

The NMR-by-treatment interaction was significant in the GEE model that included all time points (ORR=1.96; CI=(1.11, 3.46); $p=0.02$). An effect for time point (OR=0.64; CI=(0.52, 0.80); $p<0.001$) and a time point-by-treatment interaction (ORR=0.58; CI=(0.42, 0.79); $p=0.001$) indicated that varenicline's efficacy decreased significantly over time (compared to nicotine patch). We also conducted a validation analysis in which we re-ran the analyses in four different ways: Consider all dropouts to be smoking (analysis by GEE); including only cases who completed EOT, 6-, and 12-month follow-up (GEE); considering dropouts at any time-point as missing (GEE); and considering dropouts as missing data (analysis by mixed model, valid under missing-at-random dropout). The results were similar under all four models, with ratios of ORs ranging from 2.00 to 2.07, and p values ranging from 0.01 to 0.04.

As predicted, at EOT, varenicline was more efficacious than nicotine patch in NMs (OR=2.17; CI=(1.38, 3.42), $p=0.001$), but not in SMs (OR=1.13; CI=(0.74, 1.71), $p=0.56$), yielding a significant NMR-by-treatment interaction (ORR=1.89; CI=(1.02, 3.45); $p=0.04$). In a model including the placebo group as a reference, the interaction effect was similar ($p=0.05$). Of the covariates, only nicotine dependence score predicted quitting significantly (OR=0.83; CI=(0.76, 0.90); $p<0.001$). Likewise, at 6-months, varenicline was more efficacious than nicotine patch in NMs (OR=2.17; CI=(1.4, 3.4), $p=0.001$), but not in SMs (OR=1.12; CI=(0.74, 1.71), $p=0.57$); the NMR-by-treatment interaction was statistically significant (ORR=2.07; CI=(1.01, 4.22); $p=0.05$). Of the covariates, only nicotine dependence score predicted quitting significantly (OR=0.83; CI=(0.76, 0.91); $p<0.001$). The interaction effect at 12-months was not significant (ORR=1.78; CI=(0.83, 3.80); $p=0.14$). The quit rates are as follows for EOT ([SMs]: placebo 17.2%, nicotine patch 27.7%, and varenicline 30.4% versus [NMs]: placebo 18.6%, nicotine patch 22.5%, varenicline 38.5%), 6-months ([SMs]: placebo 14.4%, nicotine patch 21.6%, and varenicline 19.1% versus [NMs]: placebo 12.9%, nicotine patch 13.6%, and varenicline 22.0%), and 12-months ([SMs] placebo 13.4%, nicotine patch 19.4%, and varenicline 14.1% versus [NMs]: placebo 10.9%, nicotine patch 13.1%, and varenicline 16.0%).

The number needed to treat (NNT) was calculated using conventional methods,²⁸ contrasting nicotine patch vs. placebo, and varenicline vs. placebo, at EOT. Among NMs, nicotine patch yielded an NNT of 26.0 (CI=19.7, 32.3) while varenicline yielded an NNT of 4.9 (CI=4.7, 5.1). For SMs, the NNTs were 10.3 (CI=9.4, 11.2) and 8.1 (CI=7.5, 8.7), respectively.

The ROC analyses revealed that the area was 0.51 (95% CI=(0.43, 0.58)) for placebo, 0.54 (0.47, 0.60) for nicotine patch, and 0.54 (0.48, 0.60) for varenicline. Although effects were in the predicted direction, comparisons of ROC areas between treatment arms were not significant.

Subjective Measures

For varenicline (vs. placebo), a significant NMR-by-treatment interaction was observed in side-effects (summary from the self-report checklist; $\beta=-1.06$; CI=(-2.08, -0.03); $p=0.044$).

This reflected greater summary side-effects reported on varenicline (vs. placebo) for SMs ($\beta = 0.61$; $CI = (-0.10, 1.32)$; $p = 0.09$), but not for NMs ($\beta = -0.44$; $CI = (-1.19, 0.30)$; $p = 0.24$). Descriptive (post-hoc) item-level analysis indicated that, in SMs, varenicline led to significant increases in nausea ($\chi^2 = 18.7$, $p = 0.0003$) and abnormal dreams ($\chi^2 = 13.0$, $p = 0.005$); in NMs, varenicline led to significant increases in nausea ($\chi^2 = 15.7$, $p = 0.01$), but decreases in irritability ($\chi^2 = 15.4$, $p = 0.001$), anxiety ($\chi^2 = 11.2$, $p = 0.01$), and attentional disturbance ($\chi^2 = 11.3$, $p = 0.01$). For side-effects on nicotine patch (vs. placebo), the NMR-by-treatment interaction was not significant ($\beta = -0.17$; $CI = (-1.21, 0.86)$; $p = 0.74$).

SAEs, defined as any adverse event without regard to causality that resulted in death, was life-threatening, required hospitalization, or resulted in disability/incapacity, were determined by site physicians. There were 16 (3.9%), 22 (5.3%), and 11 (2.6%) SAEs on placebo, nicotine patch, and varenicline, respectively. Treatment arm effects or NMR-by-treatment interactions on SAE counts were not significant (see Supplemental Table 1 for side-effect counts by treatment and NMR group and Table 2 for summary side-effects values). There were no NMR-by-treatment interactions for withdrawal symptoms or medication adherence (P s > 0.10). On average, 62% of participants used 80% of the pill dose recommended and 63% used 80% of the patches recommended, comparable to previous reports.^{29,23}

Discussion

In this biomarker-stratified randomized clinical trial, varenicline was superior to nicotine patch for NMs, but had equivalent efficacy for SMs. SMs, but not NMs, reported more overall side effects on varenicline (vs. placebo). Although SMs were oversampled to be roughly 50% of the ITT sample, 40% of smokers attending the study intake were reclassified as SMs by the 0.31 cut point (illustrated in Supplemental Figure 1A). Thus, matching treatment choice based on the NMR could provide a viable clinical strategy for optimizing quit rates for all smokers, while minimizing side-effects for SMs.

As expected, the significant pharmacologic effect by NMR decreased after treatment was discontinued. A large decrease in quit rates on varenicline was observed over time, consistent with prior reports.¹⁷ Due to the relapsing nature of tobacco dependence, the current paradigm of short-term treatment has been challenged by clinical trials of extended duration therapy.²³ A placebo-controlled trial comparing 6-months of extended duration nicotine patch vs. the standard 8-weeks duration of nicotine patch showed that SMs achieve significant benefit from extended therapy with 6-month quit rates of ~50%.³⁰ An important question is whether NMs would benefit from extended varenicline therapy.

An improved understanding of the mechanisms underlying NMR associations with treatment response could help to refine the use of this biomarker in clinical practice. The higher quit rates with nicotine patch among SMs vs. NMs are not due to differences in plasma nicotine levels,¹³ nor are these differences due to nicotine withdrawal as shown here. Moreover, the NMR is not associated with nicotine dependence in most studies,¹⁵ and controlling for baseline cigarettes per day or dependence did not alter our findings.

Other neuropharmacological mechanisms can be considered. Because NMs smoke more cigarettes per day than SMs,³¹ conditioned smoking responses may be stronger in this group. NMs have enhanced responses in the brain's dopamine reward circuitry when viewing smoking cues, compared to SMs.³² Further, NMs have greater daily fluctuation in blood (and presumably brain) nicotine concentrations than SMs, which could contribute to greater reward from smoking.³³ This could explain why, for NMs, both varenicline and bupropion are more efficacious than nicotine patch. Although bupropion and varenicline have differing mechanisms of action, both are non-NRT medications that increase dopamine levels in brain reward pathways.

The present study had both strengths and limitations. It is the largest pharmacogenetic study of tobacco dependence treatment and the first to use prospective stratification. The rate of loss to follow-up did not differ by treatment arm or NMR group. The mixed ethnicity of participants and the absence of gender or race interactions with NMR and treatment suggest that the NMR works well in both Caucasian and African American smokers; however, a limitation is that few Hispanics or Asians were included. As in most smoking cessation trials, we excluded individuals with major psychiatric and medical comorbidities, limiting the generalizability of our findings. Lastly, the quit rates are lower than some previous studies.^{17, 23} This may be due to the high unemployment rate in our sample (37%). Tobacco use is associated with unemployment, and was reported to increase during the recent economic decline,³⁴ coinciding with our study.

With respect to the clinical implications, it is important to recognize that varenicline may be superior to the nicotine patch for smokers overall and that the side-effects of varenicline are generally mild and tolerable. Thus, varenicline could be prescribed to all smokers without consideration of NMR, especially in countries such as the United Kingdom, which do not caution use for certain subgroups of smokers. However, varenicline does have a "black-box warning" in the United States. Further, our data indicate that SMs do not achieve greater benefit from varenicline relative to nicotine patch, and are vulnerable to more side effects than NMs.

Thus, in addition to the imperative of increasing the utilization of treatments for nicotine dependence,³⁵ our data suggest that treating NMs with varenicline, and SMs with nicotine patch could provide a viable clinical approach. Extending the duration of use of these treatments could potentially sustain the benefits of tailored treatment.³⁰ The NMR is practical for clinical use because it is unaffected by the time of day of sampling, is stable at room temperature, is not dependent on time since last cigarette among *ad libitum* smokers, and is stable within a smoker over time.^{11, 20, 36} While the assay results were reported in under one week at \$50/sample, it is conceivable that a point-of-care test could be developed and implemented in clinical practice. However, using the NMR to individualize treatment may include additional costs and time. Our findings also underscore the notion that tobacco dependence is a heterogeneous condition and pharmacotherapies are not equally effective for all smokers.

Putting Research into Context

Systematic Review

In our search of PUBMED for the term “nicotine metabolite ratio” and “smoking cessation” (or “tobacco/nicotine dependence treatment”), we located five retrospective analyses of data collected from randomized trials testing associations of 3’hydroxycotinine/cotinine values with response to smoking cessation treatments. None of these studies prospectively stratified randomization to alternative treatments, nor did any include the newer medication varenicline. All five papers are cited within the manuscript.

Interpretation

Our findings showed that varenicline was superior to nicotine patch for normal metabolizers of nicotine, but had equivalent efficacy for slow metabolizers. Slow, but not normal metabolizers, reported more overall side-effects on varenicline (vs. placebo). These results support the potential clinical validity of the NMR as a biomarker to guide the choice of therapy for individual smokers. To optimize quit rates for all smokers while minimizing side-effects, our data support treating normal metabolizers (60% of smokers in the population) with varenicline, and slow metabolizers with nicotine patch.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

This work was supported by a grant from the National Institute on Drug Abuse, the National Cancer Institute, the National Human Genome Research Institute, and the National Institute on General Medical Sciences (U01-DA20830; C.L. and R.T.), funding from the Abramson Cancer Center at the University of Pennsylvania (P30 CA16520; C.L.), a grant from the Commonwealth of Pennsylvania Department of Health (C.L.), a grant from the Canadian Institutes of Health Research (CIHR TMH109787), an endowed Chair in Addiction for the Department of Psychiatry (R.T.), CAMH foundation (R.T.), the Canada Foundation for Innovation (#20289 and #16014), and the Ontario Ministry of Research and Innovation (R.T.). The Pennsylvania Department of Health disclaims responsibility for analyses, interpretations, or conclusions. Pfizer Inc. provided varenicline and placebo pills at no cost. No funder had a role in study design, collection and analysis of data, writing of the manuscript, or in the decision to submit this manuscript for review. The authors thank Andrea Troxel and Megan Singleton for their contributions to data and safety monitoring.

References

1. CDC. Quitting smoking among adults--United States, 2001-2010. *MMWR Morb Wkly Rep.* 2011; 60(44):1513–91.
2. WHO. World Health Organization Tobacco Fact Sheet (No 339). WHO Media Center; Geneva, Switzerland: 2013.
3. Eriksen, M.; Mackay, J.; Ross, H. The Tobacco Atlas. Fourth Ed.. American Cancer Society; World Lung Foundation; Atlanta, GA: New York, NY: 2012. Also available at www.TobaccoAtlas.org
4. West R, DiMarino ME, Gitchell J, McNeill A. Impact of UK policy initiatives on use of medicines to aid smoking cessation. *Tob Control.* 2005; 14(3):166–71. [PubMed: 15923466]
5. Fiore, MC.; Jaen, CR.; Baker, TB., et al. Clinical Practice Guideline. U.S. Department of Health and Human Services. Public Health Service; Rockville, MD: 2008. Treating Tobacco Use and Dependence: 2008 Update..
6. Hughes JR, Stead LF, Lancaster T. Antidepressants for smoking cessation. *Cochrane Database Syst Rev.* 2007; (1):CD000031. [PubMed: 17253443]

7. Aubin HJ, Bobak A, Britton JR, et al. Varenicline versus transdermal nicotine patch for smoking cessation: results from a randomised open-label trial. *Thorax*. 2008; 63(8):717–24. [PubMed: 18263663]
8. Kralikova E, Kmetova A, Stepankova L, Zvolaska K, Davis R, West R. Fifty-two-week continuous abstinence rates of smokers being treated with varenicline versus nicotine replacement therapy. *Addiction*. 2013; 108(8):1497–502. [PubMed: 23668486]
9. Wang C, Cho B, Xiao D, Wajsbrot D, Park PW. Effectiveness and safety of varenicline as an aid to smoking cessation: results of an inter-Asian observational study in real-world clinical practice. *Int J Clin Pract*. 2013; 67(5):469–76. [PubMed: 23574106]
10. Bough KJ, Lerman C, Rose JE, et al. Biomarkers for smoking cessation. *Clin Pharmacol Ther*. 2013; 93(6):526–38. [PubMed: 23588313]
11. Dempsey D, Tutka P, Jacob P 3rd, et al. Nicotine metabolite ratio as an index of cytochrome P450 2A6 metabolic activity. *Clin Pharmacol Ther*. 2004; 76(1):64–72. [PubMed: 15229465]
12. Ho M, Mwenifumbo J, Al Koudsi N, et al. Association of nicotine metabolite ratio and CYP2A6 genotype with smoking cessation treatment in African-American light smokers. *Clin Pharmacol Ther*. 2009; 85(6):635–43. [PubMed: 19279561]
13. Lerman C, Tyndale R, Patterson F, et al. Nicotine metabolite ratio predicts efficacy of transdermal nicotine for smoking cessation. *Clin Pharmacol Ther*. 2006; 79(6):600–8. [PubMed: 16765148]
14. Patterson F, Schnoll RA, Wileyto EP, et al. Toward personalized therapy for smoking cessation: a randomized placebo-controlled trial of bupropion. *Clin Pharmacol Ther*. 2008; 84(3):320–5. [PubMed: 18388868]
15. Schnoll RA, Patterson F, Wileyto EP, Tyndale RF, Benowitz N, Lerman C. Nicotine metabolic rate predicts successful smoking cessation with transdermal nicotine: A validation study. *Pharmacol Biochem Behav*. 2009; 92(1):6–11. [PubMed: 19000709]
16. Cahill K, Stevens S, Perera R, Lancaster T. Pharmacological interventions for smoking cessation: an overview and network meta-analysis. *Cochrane Database Syst Rev*. 2013; 5:CD009329. [PubMed: 23728690]
17. Gonzales D, Rennard SI, Nides M, et al. Varenicline, an alpha4beta2 nicotinic acetylcholine receptor partial agonist, vs sustained-release bupropion and placebo for smoking cessation: a randomized controlled trial. *JAMA*. 2006; 296(1):47–55. [PubMed: 16820546]
18. Brown R, Burgess E, Sales S, Whiteley J. Reliability and validity of a smoking timeline follow-back interview. *Psychol Addict Behav*. 1988; 12(2):101–12.
19. Heatherton TF, Kozlowski LT, Frecker RC, Fagerstrom KO. The Fagerstrom Test for Nicotine Dependence: a revision of the Fagerstrom Tolerance Questionnaire. *Br J Addict*. 1991; 86(9):1119–27. [PubMed: 1932883]
20. St Helen G, Novalen M, Heitjan DF, et al. Reproducibility of the nicotine metabolite ratio in cigarette smokers. *Cancer Epidemiol Biomarkers Prev*. 2012; 21(7):1105–14. [PubMed: 22552800]
21. Hughes JR, Keely JP, Niaura RS, Ossip-Klein DJ, Richmond RL, Swan GE. Measures of abstinence in clinical trials: issues and recommendations. *Nicotine Tob Res*. 2003; 5(1):13–25. [PubMed: 12745503]
22. Hughes JR, Hatsukami DK, Pickens RW, Krahn D, Malin S, Luknic A. Effect of nicotine on the tobacco withdrawal syndrome. *Psychopharmacology (Berl)*. 1984; 83(1):82–7. [PubMed: 6429705]
23. Schnoll RA, Patterson F, Wileyto EP, et al. Effectiveness of extended-duration transdermal nicotine therapy: a randomized trial. *Ann Intern Med*. 2010; 152(3):144–51. [PubMed: 20124230]
24. Norton EC, Wang H, Ai C. Computing interaction effects and standard errors in logit and probit models. *Stata J*. 2004; 4(2):154–67.
25. DeLong ER, DeLong DM, Clarke-Pearson DL. Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach. *Biometrics*. 1988; 44(3):837–45. [PubMed: 3203132]
26. Lachin JM. A review of methods for futility stopping based on conditional power. *Stat Med*. 2005; 24(18):2747–64. [PubMed: 16134130]

27. Chenoweth MJ, Novalen M, Hawk LW, et al. Known and novel sources of variability in nicotine metabolite ratio in a large sample of treatment-seeking smokers. *Cancer Epidemiol Biomarkers Prev.* 2014 (Epub ahead of print).
28. Cook RJ, Sackett DL. The number needed to treat: a clinically useful measure of treatment effect. *BMJ.* 1995; 310(6977):452–4. [PubMed: 7873954]
29. Liberman JN, Lichtenfeld MJ, Galaznik A, et al. Adherence to varenicline and associated smoking cessation in a community-based patient setting. *J Manag Care Pharm.* 2013; 19(2):125–31. [PubMed: 23461428]
30. Lerman C, Jepson C, Wileyto EP, et al. Genetic variation in nicotine metabolism predicts the efficacy of extended-duration transdermal nicotine therapy. *Clin Pharmacol Ther.* 2010; 87(5): 553–7. [PubMed: 20336063]
31. Rao Y, Hoffmann E, Zia M, et al. Duplications and defects in the CYP2A6 gene: identification, genotyping, and in vivo effects on smoking. *Mol Pharmacol.* 2000; 58(4):747–55. [PubMed: 10999944]
32. Tang DW, Hello B, Mroziwicz M, Fellows LK, Tyndale RF, Dagher A. Genetic variation in CYP2A6 predicts neural reactivity to smoking cues as measured using fMRI. *Neuroimage.* 2012; 60(4):2136–43. [PubMed: 22342802]
33. Sofuoglu M, Herman AI, Nadim H, Jatlow P. Rapid nicotine clearance is associated with greater reward and heart rate increases from intravenous nicotine. *Neuropsychopharmacology.* 2012; 37(6):1509–16. [PubMed: 22334123]
34. Kalousova L, Burgard SA. Unemployment, measured and perceived decline of economic resources: Contrasting three measures of recessionary hardships and their implications for adopting negative health behaviors. *Soc Sci Med.* 2014; 106:28–34. [PubMed: 24530614]
35. Kotz D, Brown J, West R. Prospective cohort study of the effectiveness of smoking cessation treatments used in the “real world”. *Mayo Clin Proc.* 2014; 89(10):1360–7. [PubMed: 25282429]
36. Lea RA, Dickson S, Benowitz NL. Within-subject variation of the salivary 3HC/COT ratio in regular daily smokers: prospects for estimating CYP2A6 enzyme activity in large-scale surveys of nicotine metabolic rate. *J Anal Toxicol.* 2006; 30(6):386–9. [PubMed: 16872570]

Figure 1. Consort Diagram of Flow of Study Participation

*A list of the reasons for participant ineligibility at phone screen is available from the authors on request. ** Indicates included in an intent-to-treat analysis. Inclusion and exclusion criteria were identical for phone and in-person eligibility assessments; participants who were ineligible or who withdrew or declined enrollment prior to randomization were not analyzed or followed. EOT= End-of-treatment. MINI=Mini-International Neuropsychiatric Interview. Between the EOT and 12-month follow-up assessments, 256 participants reported use of some form of smoking cessation medication (NRT, varenicline,

or bupropion). Reported use was not associated with 6- or 12-month cessation rates, treatment arm, or NMR group.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Figure 2. Quit Rates by Treatment Arm and NMR Group

Significant interaction for the head-to-head comparison of nicotine patch vs. varenicline in the longitudinal (GEE) model (ORR=1.96; CI=(1.11, 3.46); $p=0.02$). Placebo shown for comparison. Individual regression models at EOT, 6-months, and 12-months, respectively, were: (ORR=1.89, CI=(1.02, 3.45); $p=0.04$); (ORR=2.07, CI=(1.01, 4.22); $p=0.05$); (ORR=1.78, CI=(0.83, 3.80), NS). Individual p-values on graph correspond to regression models comparing nicotine patch to varenicline within metabolizer group.

Table 1

Baseline Demographic and Smoking History by Treatment Arm and NMR Group

Variable *	Placebo			Nicotine Patch			Varenicline		
	Slow N=215	Normal N=193	All N=408	Slow N=227	Normal N=191	All N=418	Slow N=220	Normal N=200	All N=420
Caucasian (N, %)	97 (45)	128 (66)	225 (55)	117 (52)	119 (62)	236 (56)	99 (45)	133 (66)	232 (55)
Black (N, %)	95 (44)	56 (29)	151 (37)	94(41)	57 (30)	151 (36)	107 (49)	53 (26)	160 (38)
Other (N, %)	23 (11)	9 (5)	32 (8)	16 (7)	15 (8)	32 (8)	14 (7)	14 (8)	28 (7)
Female (N, %)	83 (39)	91 (47)	174 (43)	88 (39)	94 (49)	182 (44)	88 (40)	99 (50)	187 (45)
High School (N, %)	68 (32)	57 (30)	125 (31)	78 (35)	49 (26)	127 (30)	74 (34)	61 (30)	135 (32)
Income \$50,000 (N, %)	65 (31)	79 (41)	144 (36)	82 (37)	75 (39)	157 (38)	74 (34)	71 (36)	145 (35)
Age (Mean, SD)	44 (11)	47 (11)	46 (11)	46 (11)	46 (11)	46 (11)	44 (12)	46 (12)	45 (12)
Not Employed (N, %)	79 (37)	84 (44)	163 (40)	69 (30)	71 (37)	140 (33)	88 (40)	75 (38)	163 (39)
FTND Score (Mean, SD)	5.3(1.92)	5.4 (2.00)	5.4 (2.00)	5.2 (2.00)	5.3 (1.89)	5.2 (1.90)	5.1 (2.00)	5.1 (2.02)	5.1 (2.01)
CPD (Mean, SD)	17.6 (7.0)	19.6 (8.7)	18.5 (7.9)	17.6 (7.0)	18.5 (7.0)	18.0 (7.0)	16.7 (5.4)	18.4 (6.3)	17.5 (5.9)

Notes.

* No significant differences between treatment arm; SD=Standard Deviation; FTND=Fagerström Test for Nicotine Dependence; CPD=Cigarettes per day.All data above are complete except 17 participants refused to provide data on income.

Table 2

Mean and Standard Errors for Total Side Effect Severity Index by Treatment Arm and NMR

	Placebo		Nicotine Patch		Varenicline	
	SM (N=215)	NM (N=193)	SM (N=227)	NM (N=191)	SM (N=220)	NM (N=200)
Week -1 (Pre-quit)	3.95 (0.33)	3.40 (0.31)	3.26 (0.26)	3.97 (0.34)	3.05 (0.27)	3.57 (0.30)
Week 0 (Target Quit Date)	4.22 (0.31)	4.27 (0.34)	3.98 (0.25)	4.28 (0.37)	4.68 (0.32)	4.06 (0.30)
Week 1	5.58 (0.36)	5.46 (0.38)	5.44 (0.41)	5.58 (0.33)	6.04 (0.36)	5.26 (0.37)
Week 4	5.33 (0.45)	4.93 (0.46)	4.24 (0.32)	4.52 (0.44)	4.97 (0.36)	4.39 (0.31)