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Predicting smoking abstinence with biological and self-report measures of adherence to varenicline: Impact on pharmacogenetic trial outcomes*

Annie R. Peng^a, Robert Schnoll^b, Larry W. Hawk Jr^c, Paul Cinciripini^d, Tony P. George^e, Caryn Lerman^b, and Rachel F. Tyndale^{a,e,*}

^aDepartment of Pharmacology and Toxicology, University of Toronto; 1 King's College Circle, Toronto, ON M5S 1A8, Canada

^bDepartment of Psychiatry and Abramson Cancer Center, University of Pennsylvania; 3535 Market Street, 4th Floor, Philadelphia, PA 19104, United States

^cDepartment of Psychology, State University of New York at Buffalo; 230 Park Hall, The State University of New York, Buffalo, NY 14260-4110, United States

^dDepartment of Behavioral Science, The University of Texas MD Anderson Cancer Center; 1155 Pressler St, Houston, TX 77030, United States

^eAddictions Division, Centre for Addiction and Mental Health and Division of Brain and Therapeutics, Department of Psychiatry, University of Toronto; 100 Stokes Street BGB 3288, Toronto, ON M6J 1H4, Canada

Abstract

Introduction—Adherence to pharmacotherapies for tobacco dependence, such as varenicline, is necessary for effective treatment. The relationship between varenicline adherence, determined by commonly used indirect (i.e., self-reported pill counts) and infrequently used direct (i.e.,

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*Correspondence: Rachel F. Tyndale, Department of Pharmacology and Toxicology, University of Toronto, 1 King's College Circle, Toronto, ON M5S 1A8, Canada.

Contributors

Annie R Peng led the analyses and preparation of the manuscript. Robert Schnoll, Larry W. Hawk Jr, Paul Cinciripini, and Tony P George served as site Principal Investigators, managed data collection and provided feedback on the manuscript. Caryn Lerman and Rachel F Tyndale were Principal Investigators for the original trial, obtained funding, lead the trial and data acquisition, assisted with data analysis and preparation of the manuscript. All authors have approved the manuscript.

Conflict of Interest

Rachel F Tyndale has consulted for Apotex and for Quinn Emmanuel, and is a member of several scientific advisory boards (e.g., Canadian Centre for Substance Abuse, Quitta, Health Canada (Vaping), and Brain Canada). Robert Schnoll receives medication and placebo supply free of charge from Pfizer and has consulted for Pfizer and GSK in the past. Paul Cinciripini served on the scientific advisory board of Pfizer, conducted educational talks sponsored by Pfizer on smoking cessation (2006–2008), and has received grant support from Pfizer. Larry W Hawk is conducting a varenicline trial (NCT03262662) for which Pfizer is providing varenicline and placebo at no cost. Pfizer has no other role in the trial.

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varenicline levels) methods, and abstinence outcomes have not been previously examined, nor has their impact on the outcomes of a genetically randomized clinical trial been assessed.

Methods—At Week 1 following target quit date, self-reported pill count and salivary varenicline levels were obtained from participants (N=376) in a smoking cessation clinical trial (NCT01314001). Point-prevalence abstinence was biochemically-verified by salivary cotinine at Week 1 and by exhaled carbon monoxide at Week 1, end-of-treatment, 6 and 12 months following treatment. Blood nicotine metabolite ratio (NMR) was obtained at baseline.

Results—Adherent individuals based on varenicline levels were significantly more likely to be abstinent than non-adherent individuals at Week 1 (odds ratios [ORs] 1.92–3.16, p 's .006), end-of-treatment (OR=2.53, p =.004), and six months following treatment (OR=2.30, p =.03). In contrast, pill counts did not consistently predict abstinence. Including direct measures of adherence enhanced the association between rate of nicotine metabolism (NMR) and end-of-treatment abstinence; normal metabolizers (NMR ≥ 0.31) were significantly more likely than slow metabolizers (NMR<0.31) to be abstinent at end-of-treatment (OR=2.00, p =.005).

Conclusion—Adherence based on salivary varenicline, rather than on pill counts, is predictive of Week 1 abstinence, irrespective of the biomarker of abstinence assessed, and of long-term abstinence. Direct measures of adherence enhance the ability to assess the impact of a biomarker or genetic marker on abstinence outcomes.

Keywords

varenicline; smoking cessation; treatment adherence; compliance; treatment outcome

1. Introduction

Adherence, discussed here as the extent to which an individual takes medication as prescribed by a healthcare professional, is an important contributor to positive health outcomes (Lam and Fresco, 2015). According to the World Health Organization, adherence to medication averages about 50% among patients living with chronic diseases and about 70%–80% among patients with acute treatment conditions (Bosworth et al., 2006; Sabaté and World Health Organization, 2003). As a result of medication nonadherence patients receive suboptimal clinical benefits and are more likely to experience poorer health outcomes. Reduced adherence poses a significant barrier to effective treatment of tobacco dependence (van Dulmen et al., 2007); smoking remains a major source of preventable morbidity and early mortality worldwide, responsible for 20% of deaths in men and 5% in women over the age of 30 (Jha et al., 2006; USDHHS et al., 2014). While varenicline is the most effective medication for tobacco dependence (Cahill et al., 2013; Eisenberg et al., 2008), adherence rates to varenicline decrease rapidly in the first six weeks of treatment (Solberg et al., 2010).

Greater adherence to smoking cessation pharmacotherapy is generally associated with higher smoking abstinence, however there is little known about varenicline adherence, including the concordance of the adherence measures, or the impact of adherence on abstinence (Catz et al., 2011; Hays et al., 2010; Liberman et al., 2013; Shiffman, 2007; Shiffman et al., 2008). Medication adherence is assessed through both indirect and direct

methods. A common indirect method of measuring adherence is via self-reported pill counts since the method is affordable and practical (Hollands et al., 2015; Lam and Fresco, 2015). Direct methods such as measurement of the drug and/or its metabolite in body fluids (i.e., blood, urine or saliva) are not frequently used due to greater effort, technical expertise required, and cost of sample collection and analysis (Lam and Fresco, 2015; Vermeire et al., 2001). Despite these challenges, a direct biological method is generally more accurate than pill count in assessing adherence and has been shown to be a strong predictor of smoking cessation outcomes (Vermeire et al., 2001; Zhu et al., 2012). Within a randomized placebo-controlled bupropion cessation trial, Nollen et al. (2013) reported moderate association between bupropion plasma levels and self-reported pill count. Further analyses found that levels of hydroxybupropion, the active metabolite of bupropion with long half-life and high plasma concentrations, were predictive of smoking abstinence at Weeks 3, 7 and 26 (Zhu et al., 2012). In contrast, the relationship between varenicline adherence—as determined by indirect and direct methods—and subsequent abstinence outcomes has not been previously examined. Varenicline is an excellent candidate for drug monitoring due to its twice daily dosing, minimal metabolism (i.e., 90% excreted unchanged) and long elimination half-life (i.e., approximately 24 hours, reaching steady-state within 4 days) (Faessel et al., 2010; Faessel et al., 2006).

Thus, this study examined the relationship between varenicline adherence, measured by direct and indirect methods, and abstinence outcomes over time. It aimed to compare self-reported pill counts and salivary varenicline levels: (1) with each other and with abstinence at time of measurement (Week 1) using two biomarkers and two cut-points of abstinence, and (2) as predictors of early and late abstinence. We hypothesize that salivary varenicline levels, a direct measure of adherence, will be a stronger predictor of abstinence compared to self-reported pill counts, an indirect measure of adherence. Additionally, in the parent trial focused on the nicotine metabolite ratio (NMR), a genetically informed biomarker of CYP2A6 genetic variation, there was a significant interaction between NMR and treatment efficacy. Relative to slow metabolizers ($\text{NMR} < 0.31$), varenicline was superior to nicotine replacement patch for normal metabolizers ($\text{NMR} \geq 0.31$) (ratio of odds ratios [ORR] 1.96, 95% CI 1.11–3.46; $p = .02$) (Lerman et al., 2015). In a final aim, we examine the relative impact of different measures of adherence on the impact of this NMR biomarker on varenicline assisted abstinence, hypothesizing that the effect of NMR on abstinence would be greater among those who are biologically adherent, as expected for a gene by drug pharmacogenomic outcome.

2. Methods

2.1 Participants

The current study is a secondary analysis of data collected from a placebo-controlled randomized clinical trial (NCT01314001). Treatment-seeking adults interested in quitting smoking (aged 18–65 years), who smoked at least 10 cigarettes per day for the past six months, were recruited and randomized to placebo, transdermal nicotine, or varenicline, stratified by their rate of nicotine metabolism. Exclusion criteria included current use of stimulants, anticoagulants, opiate medications, or antipsychotics; use of non-cigarette

tobacco products; history of substance misuse treatment; and medical contraindications. A detailed overview of study procedures with inclusion/exclusion criteria has been previously published (Lerman et al., 2015; Schnoll et al., 2014). The present analyses were restricted to participants randomized to varenicline treatment who provided a saliva sample for varenicline drug levels at the Week 1 visit (i.e., 14 days following varenicline treatment initiation; N=376). The placebo and transdermal nicotine (NRT) arms were not assessed given the lack of treatment drug levels (i.e., placebo arm) and possible confounded (i.e., nicotine from NRT or smoking) drug level data.

2.2 Measures

A study timeline is depicted in Figure 1. At intake, baseline demographic and smoking-related variables were collected.

2.2.1 Assessments of adherence—Varenicline was prescribed under the recommended dosing regimen (Day 1–3, 0.5 mg once daily; Day 4–7, 0.5 mg twice daily; Day 8–84, 1.0 mg twice daily). At the Week 1 assessment, saliva samples were collected for assessment of varenicline levels by LC-MS/MS (limit of quantification of <1ng/mL) (Dempsey et al., 2004; Jacob et al., 2011; St Helen et al., 2012; Tanner et al., 2015). Salivary varenicline concentration (ng/ml) is a direct measurement of Week 1 adherence which is significantly correlated to plasma varenicline levels in another study (Spearman correlation=.70, $p<.01$) (Peng et al., 2017b). A suitable cut-point for classifying adherent and non-adherent individuals was identified using Receiver Operating Characteristic (ROC) classification plots.

Self-reported pill count data, an indirect measure of adherence, was obtained at the Week 1 assessment using timeline follow-back questionnaire (Brown et al., 1998). Participants were asked to recall how many pills were taken in the 3, 7 and 14 days prior to Week 1 assessment; 100% of pills taken would be 6, 14, and 25 for 3-day, 7-day and 14-day pill counts respectively (Figure 1). Participants who reported taking 80% of the prescribed pills in the time periods were classified adherent for that time frame, as recommended (Bosworth, 2012).

2.2.2 Outcome assessments—The primary outcome was smoking abstinence at Week 1 when the adherence data were collected. Abstinence was reported (“no self-reported smoking (not even a puff) for at least 7 days before assessment”), followed by biochemical verification using exhaled CO and salivary COT. Abstinence was defined by recommended cut-points of 8ppm CO, as used in the parent trial, and 15ng/ml salivary COT (SRNT Subcommittee on Biochemical Verification, 2002). The effect of adherence was also examined using more stringent abstinence cut-points of 3ppm CO and 3ng/ml salivary COT (Benowitz et al., 2009; Javors et al., 2005). Secondary outcomes included CO-verified (8ppm) end-of-treatment (EOT, Week 11), 6 months (Week 24) and 12 Months (Week 52) abstinence.

2.2.3 Statistical analyses—Mann-Whitney U Tests (2-tailed) and Chi-Square Tests for Independence were employed to compare sample characteristics between Week 1 abstinent and non-abstinent individuals. Characteristics that differed between abstinent and non-

abstinent individuals ($p < .1$) were included as covariates in subsequent regression analyses. We examined the ability of various adherence measures to discriminate Week 1 abstinence status and obtained optimal criteria through ROC curve analyses and Youden index calculations, respectively. ROC curve analyses are utilized to assess the ability of a continuous predictor (i.e., measure of adherence) to correctly discriminate or classify an outcome (i.e., abstinence) (Hajian-Tilaki, 2013; Pencina et al., 2013; Simundic, 2009). Logistic regression analyses were conducted to examine the relationship between adherence measures and abstinence at various time points (Week 1, EOT, 6 months, 12 Months), and the relationship between the nicotine metabolite ratio (NMR) and abstinence within the varenicline arm following exclusion of non-adherent individuals; latter analyses controlled for baseline nicotine dependence (FTND), study site, gender and race as done in the parent trial (Lerman et al., 2015). Current analyses are not corrected for multiple analyses, based on the relatively small number of planned comparisons. Statistical analyses were performed using SPSS Statistics (Version 22, IBM Corporation) and MedCalc (Version 17.4).

3. Results

3.1 Sample description

421 individuals were randomized into the varenicline arm but only 376 provided a saliva sample for varenicline testing; there were no significant differences in baseline demographic or smoking-related characteristics between individuals who provided a saliva sample ($N=376$) and those who did not ($N=45$) (Suppl. Table 1*).

Baseline characteristics are shown in Table 1. At Week 1, 69% ($N=258$) of the participants were CO-verified abstinent (CO ≤ 8 ppm). Abstinent, compared to non-abstinent, individuals had significantly lower baseline nicotine dependence scores (FTND), reported consuming a greater number of alcoholic drinks per week at baseline, were more likely to be employed and possess an annual income greater than \$50,000 (Table 1). There were also significant differences in the proportion of abstinent individuals by study site. At Week 1, abstinent individuals had significantly higher salivary varenicline levels than non-abstinent individuals. In contrast, there were no significant differences in mean of 3, 7, and 14-day pill counts by Week 1 abstinence status ($p > .10$); this was similarly observed in the placebo arm of the parent trial (i.e., no difference in self-reported placebo pill count by Week 1 abstinence, $p > .20$). Week 1 CO-verified abstinent individuals were significantly more likely to be abstinent at EOT (OR=10.00, 95% CI=5.13, 19.49; $p < .001$), 6 months (OR=6.75, 95% CI=3.00, 15.15; $p < .001$), and 12 Months (OR=17.27, 95% CI=4.14, 72.00; $p < .001$).

3.2 Relationship between adherence measures and week 1 abstinence

3.2.1 Ability of adherence measures to discriminate week 1 abstinence—Week 1 salivary varenicline was the only measure of adherence that discriminated Week 1 abstinence (area under the curve [AUC] ranging from .567 to .608, p 's $\leq .025$) across different cut-points for CO and COT-verified abstinence (Figure 2a–d). In contrast, total 3, 7, and 14-

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day pill counts were not significant discriminators of Week 1 abstinence ($p's > .156$) (Figure 2a–d).

Optimal salivary varenicline levels in discriminating Week 1 abstinence were identified based on Youden index and ROC classification plots (Table 2); these salivary varenicline cut-points maximized sensitivity and specificity in discriminating abstinence from non-abstinence. As the stringency of CO-verified abstinence cut-points increased from 8ppm to 3ppm, the optimal salivary varenicline level increased from 1ng/ml (i.e., the detection limit) to 6.05ng/ml (Table 2a–b). A salivary varenicline level of 5.95ng/ml was optimal in discriminating COT-verified abstinence for both 15 and 3ng/ml cut-points.

3.2.2 Adherence measures as predictors of week 1 abstinence—We examined the ability of the four different adherence measures to predict Week 1 abstinence using four different abstinence cut-points (Figure 3). Adherence was defined as having taken 80% of the total 3, 7, or 14-day pill count (Bosworth, 2012), or having levels of salivary varenicline greater than or equal to the optimal level obtained in Table 2. Across the four different abstinence cut points, individuals classified as adherent based on salivary varenicline levels were significantly more likely to be abstinent than non-adherent individuals (ORs ranging from 1.92 to 3.16, $p's < .006$) (Figure 3a–d). Within intent-to-treat analyses (participants who did not provide a saliva sample or attend Week 1 session were considered non-abstinent), adherence based on salivary varenicline remained a significant predictor of Week 1 abstinence defined as CO of 8ppm (OR=7.49, 95% CI=4.52, 12.4; $p < .001$).

When using a traditional cut-point of 80% of pills taken in the preceding 3 or 7 days (as determined by pill count) to classify adherence (Bosworth, 2012), there were no statistical differences in Week 1 abstinence rate between adherent and non-adherent individuals (ORs ranging from 1.40 to 2.23, $p's > .05$). Adherence based on having taken 80% of the total 14-day pill count was predictive of Week 1 abstinence using the more stringent cessation cut-points (3ppm CO and 3ng/ml COT) (Figure 3b and 3d). Total 14-day pill count as a significant occasional predictor of abstinence should be taken in light of the highly significant correlations among self-reported 3, 7 and 14-day pill counts (Spearman correlations=0.75–0.86, $p < .001$) (Peng et al., 2017b). Overall, adherence based on salivary varenicline levels, and not self-reported pill counts, is the most consistent and significant predictor of Week 1 abstinence.

As an exploratory analysis, we examined whether adherence based on optimal pill count by Youden index, rather than the traditional 80% pill count cut-point, would be predictive of Week 1 abstinence (Suppl. Table 2*). The optimal cut points varied widely by both the days of pill count and abstinence indicator, as did the resulting ORs; little consistency was observed suggesting pill counts are not consistent predictors of abstinence even when optimization is attempted.

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3.3 Relationship between adherence measures and long-term abstinence

We examined whether early (i.e., Week 1) adherence measures predicted long-term abstinence using CO-verified 8ppm abstinence, as used in the parent trial (Lerman et al., 2015). Salivary varenicline adherence cut-point of 1ng/ml was examined as it was the optimal cut-point for discriminating CO-verified Week 1 abstinence (8ppm) (Table 2a). Adherent individuals based on 1ng/ml salivary varenicline were significantly more likely to be abstinent at EOT (OR=2.53, 95% CI=1.36, 4.73; $p=.004$) and 6 months (OR=2.30, 95% CI=1.06, 4.98; $p=.03$), but not at 12 Months (Figure 4). Adherent individuals based on 80% pill counts were significantly more likely to be abstinent at EOT (ORs ranging from 2.78 to 8.32, p 's $\leq .02$), but not at the follow-up time points. Overall, adherence based on either salivary varenicline or self-reported pill counts was a significant predictor of EOT abstinence; the former is predictive of abstinence up to six months following treatment.

We performed additional exploratory analyses to examine whether the relationship between pill count and abstinence outcomes improved by excluding participants with inaccurate pill count recall (N=67 excluded) (i.e., reported taking 1 or more pills within 48 hours prior to saliva collection but with undetectable salivary varenicline) (Peng et al., 2017a). A 48-hour window was chosen based on single-dose varenicline pharmacokinetic data for where we would expect detectable varenicline levels 48 hours-post dose (Faessel et al., 2006). The relationship between pill count and abstinence, and between salivary varenicline and abstinence within the remaining subset of participants with accurate pill count (N=309) did not vary significantly from the primary analyses (Suppl. Table 3^{*}).

3.4 Relationship between adherence measures, nicotine metabolite ratio and end-of treatment abstinence

In the primary analyses of this clinical trial, a treatment (NRT vs varenicline) by NMR interaction was observed (ORR=1.96, 95% CI=1.11, 3.46; $p=.02$) (Lerman et al., 2015), however the impact of NMR within the varenicline arm only was not the goal. Here we focus on the impact of adherence on the NMR effect within the varenicline arm, the only arm where we can reliably distinguish treatment drug and abstinence, and we focus these analyses on EOT abstinence as it was the primary clinical endpoint in the parent trial which is directly related to active treatment (Lerman et al., 2015). When examined among all participants randomized as intent-to-treat to the varenicline treatment arm, the differences in EOT abstinence rate by NMR was not significant (OR=1.33, 95% CI=0.88, 2.03; $p=.18$) (Figure 5). When restricting these analyses to those who were deemed adherent by detectable salivary varenicline levels, normal metabolizers (NMR ≥ 0.31) were significantly more likely than slow metabolizers (NMR < 0.31) to be abstinent at EOT (OR=2.00, 95% CI=1.23, 3.24; $p=.005$). In contrast, there were no significant differences in EOT abstinence by NMR when adherence by self-reported pill counts was used as the inclusion criteria (ORs ranging from 1.27 to 1.36, $p \geq .18$).

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4. Discussion

This study presents novel findings regarding the use of a direct biological measure versus indirect self-reported pill count measures of adherence for predicting smoking abstinence outcomes following varenicline treatment. First, salivary varenicline, but not self-reported pill counts, significantly predicted Week 1 abstinence (Table 1). Week 1 abstinence is highly predictive of long-term abstinence both here and as seen previously (Ashare et al., 2013; Chenoweth et al., 2016). Second, salivary varenicline had the highest rate of correct classification, and the only statistically significant, albeit weak, AUC value (Figure 2), across the four cut-points used for differentiating abstinence. Third, adherence based on salivary varenicline, rather than on pill counts, was a significant predictor of Week 1 abstinence using both biochemical measures of abstinence examined (i.e., CO vs. COT). Fourth, early adherence as defined by Week 1 salivary varenicline, in contrast to pill counts, was predictive of long-term abstinence up to six months following treatment. Lastly, the effect of NMR on varenicline assisted abstinence was significant among those who were adherent by salivary varenicline, but not among those who were adherent by self-report pill count measures.

Together these findings indicate that the biological measure of varenicline adherence is a more reliable predictor of abstinence outcomes than self-reported pill counts, and should be incorporated in future smoking cessation trials to identify participants who may require additional support to ensure tobacco cessation. Given the positive relationships between treatment adherence and successful clinical outcomes observed previously and again here with varenicline treatment (Lam and Fresco, 2015), and the poor relationship between measured varenicline levels and pill counts in this trial (Peng et al., 2017b), varenicline levels may be a more accurate measure of true adherence compared to self-reported pill counts.

The range of AUCs (0.57 to 0.61) observed for the discrimination of Week 1 abstinence by both direct and indirect measures of adherence are weak, with moderate sensitivity and specificity (Figure 2). This suggests that even though a direct measure of varenicline had the highest, and the only statistically significant rate of correct classification, point-in-time measures of adherence (i.e., Week 1 varenicline levels) are relatively weak discriminators of Week 1 abstinence. The variation in AUCs observed for salivary varenicline discrimination of Week 1 abstinence across the four biochemically-verified abstinent measures may be attributed to nuances between verification methods. For example, COT discriminates abstinence over a longer time frame compared to CO (7 days versus 1 day), COT has higher sensitivity and specificity in identifying tobacco use than CO, and COT is able to capture both smokeless and combustible tobacco use whereas CO captures only the latter (SRNT Subcommittee on Biochemical Verification, 2002). The differences in biochemical abstinence measures were also reflected by the variation of optimal salivary varenicline levels for discriminating abstinence (Table 2). Together this suggests salivary varenicline is a good discriminator of early (i.e., Week 1) abstinence as defined by COT, a highly sensitive and specific biomarker of cessation, and by CO.

Properly capturing adherence within a smoking cessation trial may have utility in optimizing abstinence. We found that adherence based on salivary varenicline, but not the traditionally used 80% self-reported pill count cut-point, was consistently predictive of Week 1 abstinence for both recommended and stringent cut points of CO and COT even when controlling for factors associated with Week 1 abstinence (Figure 3). Self-reported pill counts were weakly correlated with salivary varenicline levels (Spearman correlation $<.15$, $p <.05$) whereas the three self-reported pill count measures of adherence were highly correlated with each other (Spearman correlation $>.75$, $p <.001$), as previously reported (Peng et al., 2017b). Therefore, though the 80% pill count cut-point is traditionally relied on as a measure of adherence in clinical settings, our data suggest that this indirect measure is unlikely to adequately capture true adherence and thus may not be predictive of smoking abstinence (Dunbar-Jacob et al., 2010; Nieuwlaet et al., 2014). While the three self-reported pill count measures of adherence are highly correlated with each other (Spearman correlation $>.75$, $p <.001$) (Peng et al., 2017b), it is interesting that 14-day pill count predicted CO and COT-verified Week 1 abstinence (stringent cut-points) (Figure 3). This may suggest that self-reported adherence in the first seven days of varenicline initiation, prior to the target quit date, may be an important determinant of abstinence. A further indication that pill count may be a weak measure of medication adherence is that about 20% of our participants ($N=67/376$) reported taking at least one pill within 48 hours prior to saliva collection but had undetectable varenicline levels. Additionally, our analyses examining adherence based on optimized pill count cut-points showed no consistent associations between pill count and abstinence. It should be noted that as pill counts were not statistically significant discriminators of Week 1 abstinence (Figure 2), optimized pill count cut-points obtained through the Youden index may not be reliable nor accurate (Hajian-Tilaki, 2013; Simundic, 2009).

As early abstinence is highly predictive of long-term abstinence, we were interested in whether early measures of adherence were associated with long-term cessation outcomes (Ashare et al., 2013). Adherence based on salivary varenicline were predictive of abstinence up to six months following treatment while self-reported pill counts were only predictive of EOT abstinence (Figure 4). This may be attributable to underlying behavioral processes that can change over time as adherence is multi-factorial (Dunbar-Jacob et al., 2010).

While the intent-to-treat approach is the gold standard when implementing pharmacogenomics or biomarkers into pharmacotherapy treatment optimization, assessing the impact of a gene or biomarker in a gene-treatment interaction, among those taking the treatment, can provide additional mechanistic understandings of these relationships. In the primary analyses of this trial, we observed a significant NMR by treatment interaction (Lerman et al., 2015). Here we assessed only the varenicline arm, for adherence purposes, focusing on the clinical abstinence time point most closely related to drug treatment to focus on the specific gene/biomarker by environment/varenicline treatment interaction. Amongst adherent individuals in the varenicline arm, the rate of nicotine metabolism is significantly associated with EOT abstinence when adherence is defined by detectable varenicline levels and not by self-reported pill counts (Figure 5). Normal metabolizers of nicotine had significantly enhanced abstinence relative to slow metabolizers only among those demonstrated to have taken varenicline. These clinically important observations would be

lost without proper assessment of medication adherence. Thus, in pharmacogenomic trials (gene x pharmacotherapy) aimed at assessing the impact of a biomarker or gene on a drug outcome, a better understanding of this interaction can be assessed when drug adherence is confirmed.

Given the precedent of salivary drug monitoring in other fields of research and the favorable pharmacokinetic profile of varenicline, it may be practical to incorporate measures of salivary varenicline into smoking cessation trials. Salivary drug monitoring is routinely performed in certain clinical settings due to ease of collection; it is non-invasive, requires limited training and can be sampled repetitively (Ruiz et al., 2010). Salivary drug monitoring is utilized to optimize anticonvulsants such as phenobarbital, phenytoin and carbamazepine in the treatment of epilepsy (Herkes and Eadie, 1990; Patsalos and Berry, 2013). However, we recognize the difficulties surrounding the acquisition, analysis, and costs of biological samples that may hinder the implementation of biological measures of adherence in clinical trials. Possible alternatives may include incorporation of a continuous scale of adherence, multi-measure approaches, or other novel indirect measures that have had success in other fields of research (Lam and Fresco, 2015).

In this study, we presented adherence as a binary measure as per clinical practice, however use of continuous measures of adherence, versus artificially dichotomized measures, may improve power and effect sizes (DeCoster et al., 2011). This study had several limitations. First, our findings are based on a sample of treatment-seeking smokers who met specific inclusion and exclusion criteria for the parent clinical trial and are thus not necessarily generalizable to the overall smoking population. Secondly, we sampled varenicline levels at one point in time and it is therefore not representative of adherence over time; we were also unable to assess the reciprocal nature of early abstinence and adherence (i.e., which came first). Future studies should explore whether those uninterested, or unable, to quit subsequently did not take their medications, or whether those who were unable to stop smoking subsequently stopped taking their medication. Lastly, the relationship between adherence and abstinence outcomes may be confounded by longitudinal changes in either adherence or smoking behavior (i.e., relapse) that were not presently assessed.

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Highlights

- Salivary varenicline levels predict early and long-term abstinence
- Self-report pill counts do not consistently predict abstinence
- Possible varenicline level cut-points for distinguishing abstinence were examined
- Varenicline levels are better than pill counts for identifying abstinent participants
- Impact of biomarker on outcome can be better understood when adherence is confirmed

Figure 1.

Trial timeline. Participants were prescribed varenicline under the recommended 12 weeks dosing regimen (Day 1–3, 0.5 mg once daily; Day 4–7, 0.5 mg twice daily; Day 8–Week 11, 1.0 mg twice daily). Salivary varenicline and self-reported pill counts were assessed on Day 14; 100 percent of pills taken is 6, 14, and 25 for 3-day, 7-day and 14-day pill counts respectively.

Figure 2.

Area under the receiver operating characteristic curve for different measures of adherence in discriminating Week 1 abstinence. Abstinence was biochemically verified through exhaled carbon monoxide (CO) and salivary cotinine (COT) using recommended (8 ppm, 15 ng/ml) and stringent (3 ppm, 3 ng/ml) cut-points.

^aPossible ranges are: 0–6, 0–14, and 0–25 respectively (refer to Figure 1); ^b AUC = Area under the curve, CI = Confidence Interval; ^c Null hypothesis: true area = 0.5

Expired Air Carbon Monoxide (CO)

Salivary Cotinine (COT)

Figure 3.

Adherence measures as predictors of Week 1 abstinence. Abstinence was biochemically verified through exhaled carbon monoxide (CO) and salivary cotinine (COT) using recommended (8 ppm, 15 ng/ml) and stringent (3 ppm, 3 ng/ml) cut-points.

^aOR = Odds ratio; Adjusted for factors associated with Week 1 abstinence (Table 1– Income, Employment, Baseline Alcoholic Drinks per Week, Baseline FTND, Study Site, and Race), and *a priori* factors (gender, NMR-categorical); ^b CI = Confidence interval

Figure 4.

Adherence measures as predictors of long-term abstinence. Abstinence was biochemically verified through exhaled carbon monoxide (CO) recommended 8 ppm cut-point (as used in the original trial)

^aOR = Odds ratio; Adjusted for factors associated with Week 1 abstinence (Table 1– Income, Employment, Baseline Alcoholic Drinks per Week, Baseline FTND, Study Site, and Race), and *a priori* factors (gender, NMR-categorical); ^b CI = Confidence interval

Figure 5.

EOT abstinence rate by adherence measure and by nicotine metabolism status. Comparisons of End of Treatment abstinence for Slow versus Normal metabolizers among all subjects randomized to varenicline in the original trial (Lerman et al., 2015), to those considered adherent as measured by detectable varenicline levels, or 80% pill count for 3-Day, 7-day, and 14-day. ORs and P values on graph correspond to regression models comparing normal to slow metabolizers' EOT abstinence rate within each group. The models have been adjusted for nicotine dependence (baseline FTND), study site, gender and race, as done in the original trial (Lerman et al., 2015). Similar trends were observed with unadjusted regression models. Abstinence was biochemically verified through exhaled carbon monoxide (CO) recommended 8 ppm cut-point (as used in the original trial). OR=Odds Ratio, NMR = Nicotine Metabolite Ratio, NM = normal metabolizer, SM = slow metabolizer.

Table 1

Characteristics of sample at Week 1 follow-up

Characteristics	Overall ^a (N=374) N or Mean (SD)	Abstinent ^b (N=258)	Not Abstinent (N=116)	P-Value ^c
		N (% of Overall) or Mean (SD)	N (% of Overall) or Mean (SD)	
Gender				
Female	167	116 (69.5)	51 (30.5)	.947
Male	207	142 (68.6)	65 (31.4)	
Age (years)	45.3 (11.6)	44.6 (11.9)	46.7 (10.7)	.179
Race				
White/Caucasian	203	149 (73.4)	54 (26.6)	.058
Non-White	171	109 (63.7)	62 (36.3)	
Income ^d				
≤50,000/year	240	155 (64.6)	85 (35.4)	.011
>50,000/year	131	102 (77.9)	29 (22.1)	
Education				
High school or less	118 256	79 (66.9)	39 (33.1)	.647
More than high school		179 (69.9)	77 (30.1)	
Employment Status				
Employed	225	165 (73.3)	60 (26.7)	.025
Not Employed	149	93 (62.4)	56 (37.6)	
Marital Status				
Married/Living as Married	157	111 (70.7)	46 (29.3)	.619
Other	217	147 (67.7)	70 (32.3)	
Baseline Alcoholic Drinks per Week	3.2 (5.2)	3.6 (5.6)	2.1 (4.1)	.01
Baseline Cigarettes per Day	17.5 (5.9)	17.3 (5.8)	17.7 (6.2)	.583
Baseline FTND ^e	5.1 (2.0)	4.9 (2.1)	5.5 (1.8)	.006
Baseline NMR ^f				
Slow Metabolizers <0.31	202	145 (71.8)	57 (28.2)	.248
Normal Metabolizers ≥0.31	172	113 (65.7)	59 (34.3)	
Study Site ^g				
UPenn	123	76 (61.8)	47 (38.2)	.011
CAMH	84	63 (75)	21 (25)	
MD Anderson	85	52 (61.2)	32 (38.8)	
Buffalo SUNY	82	66 (80.5)	16 (19.5)	

Characteristics	Overall ^a (N=374) N or Mean (SD)	Abstinent ^b (N=258)	Not Abstinent (N=116)	P-Value ^c
		N (% of Overall) or Mean (SD)	N (% of Overall) or Mean (SD)	
Pill Count ^d				
3-Day	5.5 (1.2)	5.6 (1.1)	5.4 (1.4)	.166
7-Day	13.1 (2.3)	13.2 (2.1)	12.8 (2.7)	.102
14-Day	23.7 (2.9)	23.9 (2.6)	23.4 (3.5)	.168
Salivary Varenicline Level (ng/ml)	10.4 (12.0)	10.9 (11.3)	9.20 (13.2)	.008

^aN=374; missing Week 1 abstinence data from 2 participants;

^bAbstinence based on CO-verified point-prevalence, CO 8ppm;

^cp-values derived from Mann-Whitney U tests or Chi-Square Test for Independence (comparing abstinent against not abstinent);

^dN=371; missing data from 3 participants

^eFTND=Fagerström Test for Nicotine Dependence;

^fNMR=Nicotine Metabolite Ratio;

^gUPenn=University of Pennsylvania; CAMH=Centre for Addiction and Mental Health; SUNY=State University of New York;

^hPossible ranges are: 0–6, 0–14, and 0–25 respectively.

Table 2

Optimal salivary varenicline level in discriminating Week 1 abstinence

Optimal criterion are based on Youden index (Receiver Operating Characteristic Curve, refer to Figure 2)

Expired Air Carbon Monoxide (CO)					
Recommended Cessation Cut-Point (a) 8 ppm			More Stringent Cessation Cut-Point (b) 3 ppm		
Criterion	Sensitivity (95% CI)	Specificity (95% CI)	Criterion	Sensitivity (95% CI)	Specificity (95% CI)
1 ng/ml varenicline level (detectable)	86.43 (81.6, 90.4)	33.62 (25.1, 43.0)	6.05 ng/ml varenicline level	66.09 (58.5, 73.1)	51.00 (43.9, 58.1)
Salivary Cotinine (COT)					
Recommended Cessation Cut-Point (c) 15 ng/ml			More Stringent Cessation Cut-Point (d) 3 ng/ml		
Criterion	Sensitivity (95% CI)	Specificity (95% CI)	Criterion	Sensitivity (95% CI)	Specificity (95% CI)
5.95 ng/ml varenicline level	71.60 (64.0, 78.4)	50.93 (44.0, 57.8)	5.95 ng/ml varenicline level	72.65 (63.6, 80.5)	47.49 (41.3, 53.8)