A randomized, controlled, parallel group clinical study of cigarette smokers using an innovative oral tobacco-derived nicotine product to determine impact on cigarette consumption and biomarkers of exposure

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Research

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Abstract

Background: Despite the well-known health consequences of cigarettes, many adults still smoke. VERVE® Blue Mint Discs (Discs) are innovative, chewable, non-dissolvable oral tobacco-derived nicotine products designed for adult smokers (AS) interested in alternatives to cigarettes. We assessed impact of using Discs on product use behavior and biomarkers of exposure (BOE) in AS.

Methods: In this randomized, two-arm, parallel group study, AS (n=154) not planning to quit in the next 30 days were randomized to either a Control Group (n=62) that continued smoking their own brand cigarettes or Test Group (n=92) with the option to use Discs for four weeks under ad libitum conditions. Changes from Baseline to End of Study (EOS) between the Groups were analyzed for CPD and BOEs to select harmful and potentially harmful constituents (HPHCs) – nicotine, NNK, benzene and carbon monoxide.

Results: Most AS (78%) in the Test Group reduced their CPD (~20% on average, $p<0.05$), some (~9.4%) reducing by 50-99% and a few (~2.4%) switched completely to Discs. The changes in CPD was biochemically confirmed with significant reductions in COHb ($p<0.05$). Percent changes in BOEs for nicotine, NNK and benzene tended to be lower in participants using Discs compared to the Control Group indicating lower exposure to HPHCs.

Conclusions: Overall, our results suggest that many AS reduced their cigarette consumption, and some switched completely to Discs. Switching completely from cigarettes to Discs, if sustained over time, may offer an opportunity for smoking-related harm reduction. (238 words)

Implications

Many in public health including FDA have acknowledged a risk continuum for tobacco products wherein combustible products like cigarettes being most harmful and non-combustible products like oral tobacco products presenting lower risks. We have developed an innovative oral tobacco product that does not contain cut, ground, powdered, or leaf tobacco – a point of differentiation compared to most oral tobacco products in the U.S

This research shows that adult smokers, even those not interested in quitting, when given open access, replace their cigarettes with Discs with many reducing their cigarette consumption and some switch completely from cigarettes to Discs.

Switching completely from harmful combustible cigarettes to Discs, may present a harm reduction opportunity for those smokers unable or unwilling to quit.

Background

The harm caused by tobacco use is primarily attributable to cigarette smoking. Despite known health consequences, millions of adults are likely to continue smoking. According to the World Health Organization, there would be an estimated 1.1 billion smokers globally in 2025 [1].

Preventing smoking initiation and promoting smoking cessation are and should remain core strategies to reduce tobacco-related harm. In recent years, the public health community has increasingly recognized that providing less harmful alternatives to those smokers who are unable or unwilling to quit could be a complement to conventional tobacco control policies [2]. A general consensus is being reached in the scientific community that not all tobacco products present the same risk [3–6]. In fact, public health authorities, including FDA, agree that there is a broad continuum of risk among tobacco and nicotine products. According to this body of evidence, combustible cigarettes are most harmful while smokeless tobacco products present relatively lower risks [6]. FDA has announced that its comprehensive regulatory plan to significantly reduce tobacco-related disease and mortality will focus on striking an appropriate balance between regulation and encouraging the development of innovative tobacco products that may be less dangerous than cigarettes [7].

We have developed VERVE® Blue Mint discs (Discs) which are oral, chewable, non-dissolvable tobacco products containing approximately 1.5 mg tobacco-derived USP (United States Pharmacopeia) grade nicotine. These products are different from any other oral tobacco product on the market in that they do not contain cut, ground, powdered or leaf tobacco, but contain tobacco-derived nicotine and food or biocompatible medical grade non-tobacco ingredients including flavors.

The potential public health benefit of such products can only be achieved if they are lower risk than cigarettes and adult smokers consider them as acceptable alternatives such that they will completely switch to such products and stop smoking cigarettes altogether. Any assessment of the harm reduction potential of these products must include a determination of how consumers use the product and subsequent impact on exposure to harmful and potentially harmful constituents (HPHCs).

The purpose of this randomized clinical trial was to determine the impact of using Discs on cigarettes per day (CPD) and biomarkers of exposure (BOE) to select HPHCs in adult cigarette smokers relative to adult smokers who continued smoking and did not use Discs.

Methods

Study design

The study was an open-label, randomized, controlled, parallel group clinical study carried out in ambulatory settings at 3 sites in the US (Texas, Florida and Indiana). The main purpose of this study was to determine the impact of making Discs available to adult smokers on product use behavior and biomarkers of exposure. We also intended this to be a pilot study for a clinical model to assess the impact of open access of novel tobacco products on product use behavior and exposure to select HPHCs. The study protocol, informed consent and other relevant documents were the protocol was reviewed and approved by an Institutional Review Board (IRB, MidLands Independent IRB, Overland Park, KS). This study was conducted in accordance with Good Clinical Practice (GCP) based on the International Conference on Harmonisation (ICH) guidelines, and the corresponding sections of the US Code of Federal

Regulations (CFR) governing the Protection of Human Participants (21 CFR 50), IRBs (21 CFR 56), and the Basic Principles of the Declaration of Helsinki.

Study population

The study participants were adult cigarette smokers of 10 or more cigarettes per day (CPD) during the last six months; between 21 and 65 years of age, in generally good health and not intending to quit smoking in the next 30 days. Concomitant users of other tobacco products were not excluded from the study. Participants were excluded if they currently used nicotine replacement therapy; they had uncontrolled hypertension, history of coronary heart disease or other significant heart conditions, and/or other significant medical conditions that might interfere with study procedures; they had a history of drug or alcohol abuse within the previous 24 months; they used prescription anti-diabetic medication and/or insulin therapy within the previous 12 months; for females: they were pregnant, nursing or planning to become pregnant.

Study Products:

TEST PRODUCTS: VERVE® Blue Mint Discs are oral, non-dissolvable mint-flavored products containing approximately 1.5 mg USP (United States Pharmacopeia) grade tobacco-derived nicotine in a polymer matrix that have a firm, flexible texture which are placed in the mouth, chewed as long as the smokers want and discarded when done. These products are designed for adult smokers interested in alternative tobacco products.

OWN BRAND CIGARETTES: Participants used their own brand of cigarettes during the study.

Study Design

The study consisted of a four-week Screening Period, one-week Baseline Period, and four-week Product Use Period. Screening (Visit 1) occurred within 28 days of Day 1 (Day − 28 to Day − 1). Participants were offered smoking cessation information (Quit Assist™ brochure and website) before enrolling in the study and after exiting the study.

Participants who completed the initial evaluation were asked to use a sample of the Discs as they liked for 15 minutes and then completed a Potential Purchase Interest Questionnaire. Only participants indicating that they “definitely would buy” or “probably would buy” the product underwent the rest of the screening procedures. Participants who met the study eligibility requirements were enrolled into the study. Enrolled participants visited the study sites weekly for a study period of five weeks. The first week (Week 0) was the Baseline Period from Day 1 to Day 8 ± 1. During the Baseline Period participants used their own brand of cigarettes ad libitum and reported the amount used per day via daily Interactive Voice Response System (IVRS) tracking. At the end of the baseline period, on Day 8, participants were randomized (based on gender, daily cigarette consumption based on IVRS data [≤ 20 and > 20], and quit attempts based on Day 1 questionnaire data [any quit attempt/ no quit attempts in the past 30 days]) to the Test or Control Group. The baseline CPD was determined based on average of Day 2 through Day 8 for CPD as recorded through IVRS.

Participants in the Test Group were provided Discs and were told to use them ad libitum (up to 24 Discs per day) throughout the four-week Product Use Period, along with ad libitum use of their own brand of cigarettes. Participants in the Control Group were not provided Discs and were told they could continue to use their own brand of cigarettes ad libitum throughout the four-week Product Use Period. For the Test Group, used/unused Discs packages were collected during the weekly visits and new Discs were dispensed. Both groups reported their amount of CPD and Discs used through IVRS each day between 16:00 and 19:00. Weekly visit procedures for both groups included sample collection (urine, blood, exhaled CO) for BOE analyses and safety assessment, which included vital signs and reporting of concomitant medications and adverse events (AEs). Because this was an open label study, product use assignments were not blinded.

Study outcomes and measurements

The outcomes measured in this study were product use behavior (from daily IVRS calls) and select BOEs.

Biomarkers of Exposure

Urinary total NNAL (total 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol [NNAL]) for NNK exposure, urinary nicotine equivalents [NE, nicotine, nicotine glucuronide, cotinine, cotinine glucuronide, trans-3’-hydroxy cotinine and trans-3’-hydroxy cotinine glucuronide] for nicotine exposure, urinary S-phenylmercapturic acid [S-PMA] for benzene exposure, blood carboxyhemoglobin [COHb] and exhaled carbon monoxide [CO] for CO exposure. The BOEs measured in this study are biomarkers of exposure to smoke constituents identified by the FDA as harmful and potentially harmful constituents (HPHC). BOE assessment occurred at Visit 3 and changes were assessed at Visits 4 (after 1 week), 5 (after 2 weeks), 6 (after 3 weeks) and 7 (after 4 weeks, end of study).

Fagerström Test for Cigarette Dependence (FTCD) scores, number of quit attempts (How many times during the past 30 days have you stopped smoking cigarettes for 24 hours or longer because you were trying to quit?) and quitting intentions (Are you planning to quit smoking in the next 30 days?) were also assessed at the beginning and end of the study.

Bioanalytical methods

Urine BOEs were analyzed in spot urine samples (collected during afternoon study visits) using validated analytical methods with appropriate quality controls according to the FDA Guidance for Industry: Bioanalytical Method Validation (May 2001) and in accordance with FDA Good Laboratory Practice regulations (Title 21 CFR Part 58). The analytical measurements were conducted using liquid chromatography–tandem mass spectrometry (LC-MS/MS) described elsewhere [8]. Baseline Urine creatinine and blood COHb were analyzed at a Clinical Laboratory Improvement Amendments of 1988 (CLIA-88) certified clinical laboratory.

Nicotine equivalents (mg) were calculated as the molar sum of nicotine, total cotinine, and total trans-3'-hydroxycotinine excreted in the afternoon spot urine sample. Urinary NE, Total NNAL and S-PMA were

normalized for creatinine concentration. Exhaled CO measurement was performed with Micro + ™ Smokerlyzer® CO monitors.

Product use behavior

Daily product use was assessed by the IVRS throughout the study. IVRS is an entirely automated tool which uses a telephone as a device to input information. Study participants were instructed to call into a pre-specified number daily between 16:00 and 19:00 and answer recorded questions on their past 24-hour product use by using the number pad on a touch tone phone. Data was summarized weekly for comparisons.

Statistical analyses

Demographic baseline characteristics were summarized by study group with descriptive statistics. Fisher's exact test was used to test for the differences in categorical variables and t test to test for the differences in continuous variables.

The primary analysis was conducted in the per-protocol (PP) set (N = 142), which comprised all randomized participants who completed post-baseline assessments, had no major protocol deviations (missed clinic visits or visits outside of protocol allowed time frame), and did not discontinue from the study. A linear mixed model for repeated measures analysis of variance (ANOVA) was used for the analysis of the absolute and percent change from baseline in all BOEs. The BOE baseline values were determined based on measurements made on Day 8, baseline CPD was determined based on average of Day 2 through Day 8 for CPD as recorded through IVRS. SAS procedure Proc Mixed was used for the statistical computing.

Differences in Least Squares (LS) means between Test and Control groups were reported along with 95% CIs of the difference for each visit with P-values ($\alpha = 0.05$) of a 2-tailed t-test obtained from Mixed Effects Repeated Measures Model. The same model and statistical comparisons were used percent change from baseline in CPD and Total FTCD scores (without interaction effects).

Statistical analyses on the differences in proportion of participants in subgroups (change from baseline in weekly average of CPD, total scores of FTCD, quit attempts and quit intentions) were carried out using the Mantel-Haenszel Chi-Square test and/or Fischer's Exact Test.

Results

154 adult cigarette smokers were randomized based on gender, CPD (≤ 20 and > 20) and quit attempts (any quit attempt/no quit attempts in the past 30 days) to either the Test (n = 92) or Control Group (n = 62) in ~ 3:2 ratio. Of these 154 participants, 142 completed the study and were included in the per-protocol population.

Some participants (n = 12) were excluded from the per-protocol population due to major protocol deviations (missed visits or visits outside of protocol allowed time frame) or discontinuing from the study. This resulted in a per-protocol assessment of 87 participants in the Test Group and 55 participants in the Control Group.

There were no statistically significant differences between the study groups in demographic characteristics. The study population was comprised of n = 76 (49.4%) male and n = 78 (50.6%) female participants, with a mean age of 43 years. One (0.6%) subject was Asian, 26 (16.9%) were Black or African American, 119 (77.3%) were White, and 8 (5.2%) were of another race. Nine (5.8%) participants were Hispanic or Latino. Mean BMI was 28.6 kg/m^2. Majority of the participants (71.4%) consumed ≤20 CPD at baseline. The Test Group baseline mean CPD by IVRS at Baseline was 17.8 (+/-5.58 SD) and the Control Group mean CPD was 19.5 (+/-7.39 SD).

Product use behavior

CPD and Disc use

The statistical analyses of the differences in percent change from baseline in mean CPD between study groups is presented in Table 1. For the Test Group, mean CPD decreased at all post-baseline visits ranging from 15% to ~ 19%, while no significant change was noted for the Control Group (95% confidence interval included zero). The difference between study groups was statistically significant at all post-baseline visits, with p-values < 0.0001 (Table 1). Approximately 78% of the study participants reduced their cigarette consumption.

Table 1

Percent Change in Cigarettes Per Day

Timepoint	N[1] Test/ Control	LS Mean[2] (95% CI) Test Group % Change	LS Mean (95% CI) Control Group % Change	Difference of LS Means (Test-Control) and 95% CI for the Difference[3]	P-value[4]
Week 1	87/55	-15.0 (-18.5, -11.5)	3.1 (-1.3, 7.5)	-18.1 (-23.8, -12.5)	< 0.0001
Week 2	87/55	-16.5 (-21.2, -11.7)	2.9 (-3.1, 9.0)	-19.4 (-27.1, -11.7)	< 0.0001
Week 3	87/55	-16.3 (-21.1, -11.6)	2.9 (-3.1, 8.9)	-19.2 (-26.9, -11.6)	< 0.0001
Week 4	85/55	-19.3 (-24.3, -14.3)	3.2 (-3.1, 9.4)	-22.5 (-30.5, -14.5)	< 0.0001
[1] N was the number of participants.					
[2] Least Squared (LS) means from Mixed Effects Repeated Measures Model: Mixed Effects Repeated Measures Percent Change from baseline = study group + visit + study group*visit + subject + random error, fitted with unstructured covariance matrix; Baseline values represent an average of IVRS data collected from Days 1–8 (Week 0)					
[3] 95% confidence interval for the difference of least squares means.					
[4] P-value from two-tailed t-test obtained from Mixed Effects Repeated Measures Model.					

The mean daily consumption of Discs in the Test Group was approximately 5 discs per day (Week 1: 4.5 (SD+/2.87), Week 2: 4.9 (SD+/-3.82), Week 3: 4.6 (SD+/-3.72), Week 4: 4.9 (SD+/-3.93).

Other tobacco products were used only occasionally by a small number of participants.

Proportion of participants in subgroups based on change from baseline CPD

Participants were grouped based on changes from baseline in the weekly average of CPD (no change, < 50% reduction, 50–99% reduction, 100% reduction, any increase). The proportion of participants in the cigarette use subgroups differed statistically significantly ($p < 0.05$) between study groups at all post-baseline visits.

At the end of the study (Week 4), ~ 66% of participants in the Test Group reduced their cigarette consumption by < 50%; ~9.4% reduced their cigarette consumption by 50%-99% and ~ 2.4% did not report cigarette use for the past 7 days, the remaining participants either increased (~ 13%) or did not change (9%) their CPD (Table 2). In the Control group ~ 44% reduced their cigarette consumption by < 50%; however, none of the participants reduced their cigarette consumption by > 50% or completely stopped smoking. We note that the Disc consumption was higher (~ 9 Discs per day) in the Test Group participants that reduced CPD consumption by > 50%. Additionally, the baseline CPD in the Test Group was much lower in those individuals that switched completely to Discs and stopped smoking relative to other subgroups (Table 2).

Table 2

Distribution of Participants by Study Group Based on CPD

	Test Group				**Control Group**		
Group CPD Changes	N (% group)	CPD Baseline Mean (SD)	CPD EOS Mean (SD)	Disc use per day EOS Mean (SD)	N (% Group)	CPD Baseline Mean (SD)	CPD EOS Mean (SD)
Increase	11 (12.94)	16.2 (5.56)	17.9 (4.87)	5.1 (3.87)	29 (52.73)	19.4 (8.41)	21.6 (8.78)
No Change	8 (9.41)	17.4 (8.38)	17.4 (8.38)	4.0 (4.25)	2 (3.64)	20.0 (0)	20.0 (0)
< 50% Reduction	56 (65.88)	18.4 (5.06)	15.2 (4.1)	4.5 (3.05)	24 (43.64)	19.5 (6.49)	17.8 (5.61)
50–99% Reduction	8 (9.41)	18.5 (6.04)	5.0 (3.32)	8.6 (6.78)	0	NA	NA
100% Reduction	2 (2.35)	11.3 (2.80)	0	5.4 (7.58)	0	NA	NA

CPD = cigarette per day; Baseline = average of Day 2 to 8 for CPD as measured through IVRS; EOS = end of study;

NA = Not Applicable, SD = Standard Deviation

Data shown as LS Means, error bars represent upper limit of the 95% Confidence Interval.

* p-value < 0.05; ** p-value < 0.0001 from two-tailed t-test obtained from Mixed Effects Repeated Measures Model; CPD = Cigarettes per day, NE = Urinary nicotine equivalents (nicotine + five metabolites), NNAL = Total urinary NNAL, S-PMA = S-phenyl mercapturic acid, CO = carbon monoxide, COHb = Blood Carboxyhemoglobin.

Data for study participants that reduced cigarette consumption by 50–99% are shown as mean ± SEM. Individual observations are presented for each of the participants that stopped smoking. Dotted lines represent nonuser levels in carboxyhemoglobin based on Roethig et al., 2009 [21].

Biomarkers of exposure

The statistical analyses of the differences in percent change from baseline in mean BOEs between study groups is presented in Fig. 1.

All the BOEs levels were lower in the Test Group compared to the Control Group (Fig. 1), however significant variability was observed in all the spot urine BOE measurements (% coefficient of variation ranging from 72–99% for total NNAL, 61–81% for NE and 94–102% for S-PMA in the Test Groups). The EOS reductions were 12.6% for total NNAL, 9.8% for NE, 19% for S-PMA, 10% for COHb and 12% for eCO. Statistically significant reduction ($p < 0.05$) was observed for COHb at all measurements (Weeks 1–4), at Week 2 for eCO and S-PMA. In general, the urinary BOE levels trended higher in both Test and Control groups relative to baseline but not the COHb or eCO levels. There was a relatively higher variability observed within the groups for eCO relative to that observed for COHb.

The magnitude of reductions in BOEs in the Test Group participants that reduced their CPD 100% confirmed no smoking – eCO up to 86%, COHb up to 69%, NE up to 100%, NNAL up to 89%, and S-PMA up to 95%. The trajectory of change in COHb (Fig. 2 (B)) followed the trajectory for reduction in cigarette consumption. A gradual reduction in COHb was noted in the participants that reduced CPD by 50–99% at EOS and appeared to correspond with the reduction in CPD (Fig. 2). Due to the large variability observed in the spot urine measurements, no inferences can be drawn related to urinary BOE for the subgroup that reduced CPD by 50–99%.

FTCD, quit intentions and quit attempts.

There was no statistically significant difference in percent change from baseline to EOS in total score on the FTCD between study groups. Additionally, no statistically significant differences were observed between Test and Control regarding the proportion of participants indicated a change in quit intentions or quit attempts. However, 12 participants (13.95%) in the Test Group and 5 (10.00%) in the Control Group indicated intention to quit in the next 30 days at EOS relative to lack of such intentions at baseline.

Adverse events

There were no deaths or serious AEs during the study, 25 out of 92 participants (27.2%) in the Test Group reported a total of 41 AEs and 7 out of 62 (11.3%) participants in the Test Group reported 17 AEs. Majority of AEs in the Test Group (39/41 events) were mild in severity and 14/41 were determined by the Study Principal Investigator as possibly, likely or related to the test products (all considered as mild in severity). The adverse events considered product related were: cough (5 events), headache (2 events) oropharyngeal pain (2 events), dry throat (1 event), nasal congestion (1 event), throat irritation (1 event), diarrhea (1 event) and dizziness (1 event).

Discussion

We report that under the conditions of this study, when presented with the option of using Discs, many adult smokers replace their cigarettes, ~ 78% of the study participants reducing their cigarette consumption, and ~ 10% of the study participants reduced their cigarette consumption by 50% or more. These observations are of relevance since the adult smokers in this study had no intentions to quit smoking in the next 30 days. Further, as illustrated by the BOE measurements, adult tobacco consumers replacing cigarettes with the candidate products have lower exposure to the HPHCs found in tobacco smoke.

While the maximum health benefit can best be achieved by smoking cessation, smoking reduction is a potential pathway to smoking cessation [9–14]. No scientific consensus exists among public health regarding the impact of smoking reduction and relationship to disease risks. Favorable changes in cardiovascular risk markers and quality of life assessments have been reported from long-term smoking reduction of at least 50% of daily cigarettes [9]. Rennard et al. [15] report that smoking reduction with the help of nicotine gum was associated with decreased respiratory tract inflammation as assessed by bronchoalveolar lavage. While smoking reductions may have favorable health outcomes, smoking cessation is the desirable outcome to achieve proven reductions in smoking-related disease risks. Nevertheless, the reductions in BOE (nicotine, NNK, benzene, and carbon monoxide) among participants replacing their cigarettes with Discs indicates lower exposure to HPHCs, which is encouraging, and indeed a small number of participants switch completely from cigarettes to Discs. The average reduction in blood COHb corresponded to the CPD reduction for the overall study population (~ 20% CPD reduction), in the 50–99% CPD reduction group as well as for the participants reducing their CPD by 100%, thus providing biochemical confirmation of CPD reduction.

Further assessment revealed that one participant completely switched to the Discs and the other participant stopped using all tobacco products. The reductions in the BOE for HPHCs including NNK, benzene and carbon monoxide exposure confirmed the changes in product use behavior in these two participants. For the participant who reported complete tobacco product cessation, we did not detect nicotine levels and the reductions in urinary NNAL were comparable to the estimated levels expected upon smoking cessation. These results further verified the self-report status of no cigarette consumption for both study participants. While these numbers are small, such changes in cigarette consumption were not observed in the Control Group.

Although all the urinary BOEs tended to be lower in the Test Group compared with the Control Group, they did not correspond to the extent of CPD reduction which could be due to three likely reasons. First, as reported by Hatsukami et al [16] reduction in cigarette consumption may be accompanied with compensatory cigarette smoking behavior. However, the proportionate reduction in the blood COHb levels does not support likely occurrence of this phenomenon. Second, the dose-response relationship between CPD and BOEs differs among BOEs and the elimination kinetics may influence the biomarkers' ability to reflect changes in cigarette consumption. In particular, NNAL has a half-life of 10- to 18-days and can be detected in urine for 6 to 12 weeks after smoking cessation [17], thus requiring similar time to reach a new steady state level after switching between tobacco products. Third, variability in spot urine is often

observed, even after adjusting for creatinine [18, 19]. Circadian rhythm in glomerular filtration rate (GFR) and renal plasma flow has been reported in healthy individuals [20]. Day-time GFR was found to be higher compared with overnight, and urine flow was found to have the same circadian rhythm as GFR. Within individual variability in biomarkers (e.g. cadmium excretion ranging from 4-10-fold) has been attributed to circadian rhythm. Similarly significantly high variability has been observed in cigarette smoke related BOE during day-time urine measurements than early morning voids [19]. We collected spot urine samples during afternoon visits to the study site, perhaps the large variability in spot urine samples may be minimized in first morning void samples.

The study results should be interpreted with the limitations of the study. The participants received the test products free. Thus, only limiting inferences can be drawn regarding actual product use behavior under “real-world” conditions since the study does not consider all the factors that may influence purchase decisions of adult smokers. Such limitations may be offset by conducting in-market observational studies. The number of participants switching to Discs and reducing cigarette consumption by > 50% was relatively small (~ 12%). However, given that none of the AS in the Control Group exhibited this behavior and that the participants did not intend to quit, this phenomenon suggests that the impact of Discs might alter smoking behavior in a favorable direction. Recall bias from tracking test product use under ambulatory conditions is another limitation, however likely similar recall bias in the control group and daily IVRS calls may counterbalance this limitation. Another potential limitation is that this study was not designed as a complete switching study and concomitant use of other tobacco products was allowed. Although these products were reportedly used only sporadically by a small number of participants, their impact on BOEs has not been specifically analyzed and cannot be ruled out. The participants enrolled in the study were only those that expressed an interest in using the product upon a single use trial, thus likely incorporating self-selection bias. Nevertheless, this might reflect a realistic situation where an adult smoker may try a new product before making a decision to become a regular user. The clinical model of presenting adult smokers with the open access and choice of using a new tobacco product is a useful paradigm to assess the impact on product use behavior and exposure to select HPHCs.

Conclusion

Overall, administration of Discs was well tolerated over the 4-week product use period in this population of healthy male and female smokers. Discs may be considered as a potential replacement for cigarettes and Disc use can help smokers not intending to quit to reduce their cigarette consumption that might eventually lead to complete switching to Discs accompanied with no cigarette consumption.

Abbreviations

AE	adverse event
ANOVA	analysis of variance
BMI	body mass index
BOE	biomarker of exposure
CFR	Code of Federal Regulations
CI	confidence interval
CO	carbon monoxide
COHb	blood carboxyhemoglobin
CPD	cigarettes per day
CV	coefficient of variation
FDA	Food and Drug Administration
FTCD	Fagerström Test for Cigarette Dependence
GCP	Good Clinical Practice
ICF	informed consent form
IRB	Institutional Review Board
IVRS	interactive voice response system
max	maximum
min	minimum
N, n	number
NE	nicotine equivalent
NNAL	4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol
SAE	serious adverse event
SD	standard deviation
S-PMA	S-phenylmercapturic acid
USA	United States of America

Declarations

- *Ethics approval and consent to participate*

The study protocol, informed consent and other relevant documents were the protocol was reviewed and approved by an Institutional Review Board (IRB, MidLands Independent IRB, Overland Park, KS). This study was conducted in accordance with Good Clinical Practice (GCP) based on the International Conference on Harmonisation (ICH) guidelines, and the corresponding sections of the US Code of Federal Regulations (CFR) governing the Protection of Human Participants (21 CFR 50), IRBs (21 CFR 56), and the Basic Principles of the Declaration of Helsinki.

- *Consent for publication*

See attached Consent Forms from all the authors

- *Availability of data and material*

The datasets generated and/or analyzed during the current study are not publicly available as we have submitted an application to FDA for market authorization of the product. We will consider reasonable requests for underlying data after a decision from FDA.

- *Competing interests*

Jeff Edmiston, Qiwei Liang, Jianmin Liu and Mohamadi Sarkar were employees of Altria Client Services LLC, during the period of study conduct, analysis and development of this manuscript.

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N/A

- *Author's contributions*

All authors contributed towards designing the study. QL was the lead statistician supervising the data analysis, JL was the medical monitor for the study. JE and MS wrote the manuscript.

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References

1. Bilano V, Gilmour S, Moffiet T, d'Espaignet ET, Stevens GA, Commar A, Tuyl F, Hudson I, Shibuya K: **Global trends and projections for tobacco use, 1990-2025: an analysis of smoking indicators from**

the WHO Comprehensive Information Systems for Tobacco Control. *Lancet* 2015, **385**(9972):966-976.

2. Physicians RCo: **Nicotine without smoke: Tobacco Harm Reduction**. In. RCP, London; 2016.
3. Abrams DB, Glasser AM, Pearson JL, Villanti AC, Collins LK, Niaura RS: **Harm Minimization and Tobacco Control: Reframing Societal Views of Nicotine Use to Rapidly Save Lives**. *Annu Rev Public Health* 2018, **39**:193-213.
4. Abrams DB, Glasser AM, Villanti AC, Pearson JL, Rose S, Niaura RS: **Managing nicotine without smoke to save lives now: Evidence for harm minimization**. *Prev Med* 2018, **117**:88-97.
5. Nutt DJ, Phillips LD, Balfour D, Curran HV, Dockrell M, Foulds J, Fagerstrom K, Letlape K, Milton A, Polosa R *et al*: **Estimating the harms of nicotine-containing products using the MCDA approach**. *Eur Addict Res* 2014, **20**(5):218-225.
6. Zeller M, Hatsukami D, Harm_Reduction_Group: **The Strategic Dialogue on Tobacco Harm Reduction: a vision and blueprint for action in the US**. *Tob Control* 2009, **18**(4):324-332.
7. FDA US: **FDA announces comprehensive regulatory plan to shift trajectory of tobacco-related disease and death**. In.; July 28, 2017.
8. Sarkar M, Liu J, Koval T, Wang J, Feng S, Serafin R, Jin Y, Xie Y, Newland K, Roethig HJ: **Evaluation of biomarkers of exposure in adult cigarette smokers using Marlboro snus**. *Nicotine Tob Res* 2010, **12**(2):105-116.
9. Bolliger CT, Zellweger JP, Danielsson T, van Biljon X, Robidou A, Westin A, Perruchoud AP, Sawe U: **Influence of long-term smoking reduction on health risk markers and quality of life**. *Nicotine Tob Res* 2002, **4**(4):433-439.
10. Hughes JR: **Reduced smoking: an introduction and review of the evidence**. *Addiction* 2000, **95 Suppl 1**:S3-7.
11. Hughes JR, Cummings KM, Hyland A: **Ability of smokers to reduce their smoking and its association with future smoking cessation**. *Addiction* 1999, **94**(1):109-114.
12. Klemperer EM, Hughes JR: **Does the Magnitude of Reduction in Cigarettes Per Day Predict Smoking Cessation? A Qualitative Review**. *Nicotine Tob Res* 2016, **18**(1):88-92.
13. Klemperer EM, Hughes JR, Naud S: **Reduction in Cigarettes Per Day Prospectively Predicts Making a Quit Attempt: A Fine-Grained Secondary Analysis of a Natural History Study**. *Nicotine Tob Res* 2018.
14. Schauer GL, Malarcher AM, Babb SD: **Gradual reduction of cigarette consumption as a cessation strategy: prevalence, correlates, and relationship with quitting**. *Nicotine Tob Res* 2015, **17**(5):530-538.
15. Rennard SI, Daughton D, Fujita J, Oehlerking MB, Dobson JR, Stahl MG, Robbins RA, Thompson AB: **Short-term smoking reduction is associated with reduction in measures of lower respiratory tract inflammation in heavy smokers**. *Eur Respir J* 1990, **3**(7):752-759.
16. Hatsukami DK, Le CT, Zhang Y, Joseph AM, Mooney ME, Carmella SG, Hecht SS: **Toxicant exposure in cigarette reducers versus light smokers**. *Cancer Epidemiol Biomarkers Prev* 2006, **15**(12):2355-2358.

17. Goniewicz ML, Havel CM, Peng MW, Jacob P, 3rd, Dempsey D, Yu L, Zielinska-Danch W, Koszowski B, Czogala J, Sobczak A *et al*: **Elimination kinetics of the tobacco-specific biomarker and lung carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol**. *Cancer Epidemiol Biomarkers Prev* 2009, **18**(12):3421-3425.
18. Akerstrom M, Barregard L, Lundh T, Sallsten G: **Variability of urinary cadmium excretion in spot urine samples, first morning voids, and 24 h urine in a healthy non-smoking population: implications for study design**. *J Expo Sci Environ Epidemiol* 2014, **24**(2):171-179.
19. Sarkar M, Muhammad-Kah R, Liang Q, Kapur S, Feng S, Roethig H: **Evaluation of spot urine as an alternative to 24h urine collection for determination of biomarkers of exposure to cigarette smoke in adult smokers**. *Environ Toxicol Pharmacol* 2013, **36**(1):108-114.
20. Koopman MG KG, Krediet RT, de Moor EA, Hoek FJ, Arisz L.: **Circadian rhythm of glomerular filtration rate in normal individuals.** *Clin Sci (Lond)* 1989, **77**:105-111.

Figures

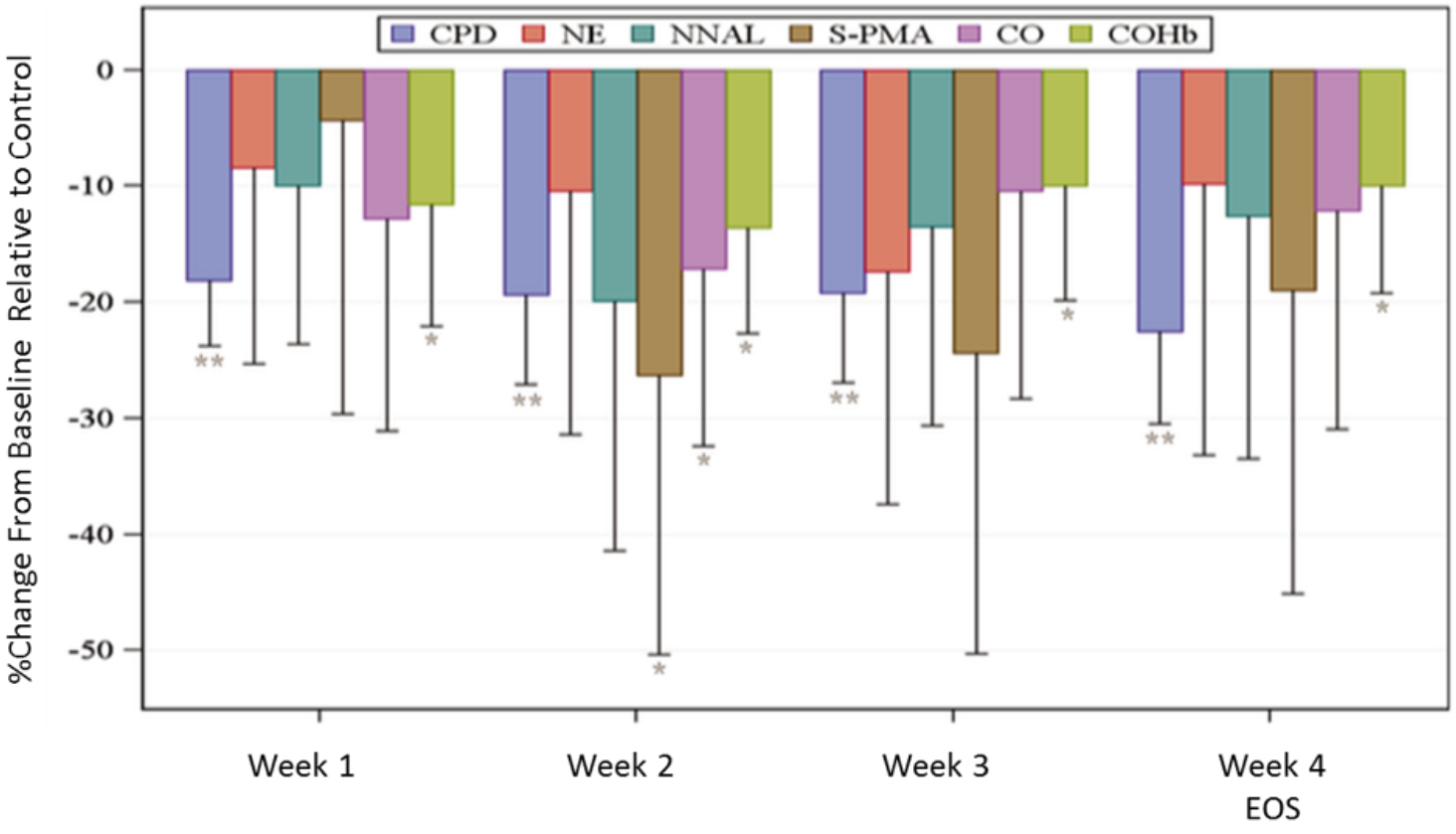

Figure 1

Group Differences in Mean Percent Change from Baseline (Test – Control) Data shown as LS Means, error bars represent upper limit of the 95% Confidence Interval. * p-value < 0.05; ** p-value < 0.0001 from two-tailed t-test obtained from Mixed Effects Repeated Measures Model; CPD=Cigarettes per day, NE=Urinary nicotine equivalents (nicotine + five metabolites), NNAL=Total urinary NNAL, S-PMA=S-phenyl mercapturic acid, CO=carbon monoxide, COHb=Blood Carboxyhemoglobin.

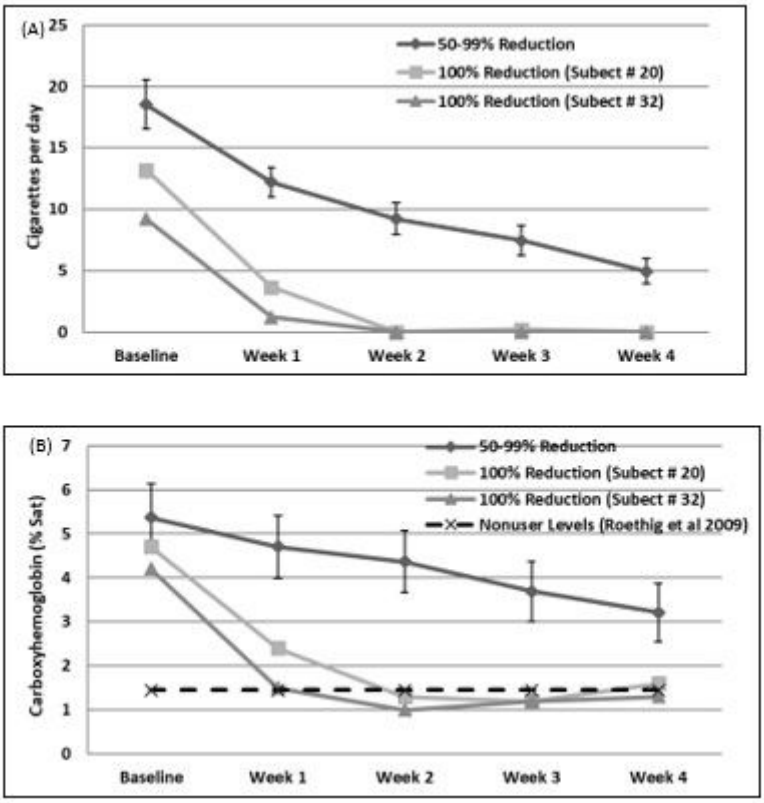

Figure 2

Reduction in Cigarettes per Day (A) and Corresponding Reduction in Carboxyhemoglobin (% saturation) (B) (Dotted lines represent nonuser levels in carboxyhemoglobin based on Roethig et al [21] Data for study participants that reduced cigarette consumption by 50-99% are shown as mean + SEM. Individual observations are presented for each of the participants that stopped smoking. Dotted lines represent nonuser levels in carboxyhemoglobin based on Roethig et al., 2009 [21].