Research Article

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Effects of Switching to a Heat-Not-Burn Tobacco Product on Biologically Relevant Biomarkers to Assess a Candidate Modified Risk Tobacco Product: A Randomized Trial

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Abstract

Background: Cigarette smoking increases the risk of chronic diseases; heating instead of burning tobacco can lower these risks, contributing to tobacco harm reduction. This study (with 984 adult American smokers) examined whether favorable changes occur in 8 co-primary endpoints (HDL-C, WBC, FEV_1%pred, COHb, Total NNAL, sICAM-1, 11-DTX-B2, 8-epi-PGF2α) indicative of biological and functional effects when cigarette smokers switch to the heat-not-burn Tobacco Heating System 2.2 (THS). Additionally, these biomarkers of exposure (BoExp) were quantified: MHBMA, 3-HPMA, Total NNN, CEMA, 3-OH-B[a]P, HMPMA, Total 1-OHP, NEQ, and CO exhaled.

Methods: Participants were randomized to continued smoking of their preferred cigarette brand ($n = 496$) or to using THS (IQOS brand; $n = 488$) for 6 months. THS has a maximum heating temperature of 350°C, delivering 1.21 mg nicotine/stick and 3.94 mg glycerin/stick under the Health Canada Intense smoking regimen.

Results: The main outcome was a favorable change 6 months after baseline, with statistically significant improvements in 5 of 8 biomarkers of effect (HDL-C, WBC, FEV_1%pred, COHb, Total NNAL) when smokers switched to THS compared with those who continued to smoke cigarettes. Likewise, BoExp were markedly reduced.

Conclusions: All endpoints showed favorable changes in the same direction as with smoking cessation and improved biological effects were observed in smokers who predominantly used THS compared with continued cigarette smoking, with similar nicotine levels in both groups.

Impact: Improvements in 5 of 8 biomarkers of effect are supportive of the research hypothesis, suggestive of disease risk reduction potential for smokers switching to THS instead of continuing to smoke cigarettes.

Introduction

Cigarette smoking is the leading cause of preventable diseases in the United States, accounting for more than 480,000 smoking-related deaths every year and more than 16 million people living with a smoking-related disease (1). Consequently, tobacco control has been the dominant strategy for reducing the smoking-related burden of disease, the underlying ambition being a tobacco-free society. Although smoking prevalence in the United States has declined from 21% to below 16% over the last decade, an estimated 38 million Americans currently smoke cigarettes (2).

Tobacco harm reduction, a complementary approach to tobacco control, is increasingly being adopted. It rests on the notion of a "continuum of risk," building on the insight that "people smoke for the nicotine but die from the tar" (3). Harm reduction can, in principle, be achieved when cigarettes are replaced by alternative nicotine delivery systems. In the United States, the Family Smoking Prevention and Tobacco Control Act authorized the U.S. Food and Drug Administration (FDA) to control the marketing of novel tobacco products, including the issuance of reduced exposure and reduced risk market orders, based on evidence provided by the applicant.

In recent years, numerous electronic nicotine delivery systems (ENDS) have been developed as alternatives to cigarettes, of which electronic cigarettes (e-cigarettes) are the best-known class of products. As a new generation of e-cigarettes are marketed with improved nicotine delivery, the use of these devices has begun to spread (4). In principle, these products aim to deliver nicotine while avoiding exposure to the combustion byproducts typical for cigarettes, which burn tobacco at temperatures in excess of 600°C. At such high temperatures, the tobacco burns and generates smoke that contains a complex mixture of more than 6,000 chemicals (5). Public health authorities have classified approximately 100 of these smoke constituents as likely

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Note: Supplementary data for this article are available at Cancer Epidemiology, Biomarkers & Prevention Online (http://cebp.aacrjournals.org/).

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causes of smoking-related diseases, such as lung cancer, heart disease, and emphysema (6).

An alternative approach to effectively deliver nicotine, taste, and a sensory experience comparable to cigarettes is heating instead of burning tobacco. The most prevalent "heat-not-burn" product to date is the Tobacco Heating System 2.2 (THS), which heats tobacco at a temperature that does not initiate combustion, and has been marketed in more than 40 countries since 2014 (brand name IQOS). Philip Morris International (PMI) has submitted a Modified Risk Tobacco Product (MRTP) Application for THS under the Federal Food, Drug, and Cosmetic Act (https://www.fda.gov/TobaccoProducts/Labeling/MarketingandAdvertising/ucm546281.htm). A Premarket Tobacco Product Application (PMTA) to market IQOS in the United States was approved by the FDA on April 30, 2019 (https://www.fda.gov/news-events/press-announcements/fda-permits-sale-iqos-tobacco-heating-system-through-premarket-tobacco-product-application-pathway).

THS consists of 3 components: (i) a tobacco stick (or *HeatStick*)—a novel patent-pending tobacco product, (ii) a holder that heats the tobacco stick by means of an electronically controlled heating blade, and (iii) a charger that is used to recharge the holder after each use. To operate THS, the user inserts a tobacco stick into the holder and turns on the device by means of a switch. This activates the heating blade inserted into the tobacco stick. The tobacco stick is heated for a duration of approximately 6 minutes and allows up to 14 puffs to be taken during that time. The temperature of the heating blade is carefully controlled and the energy supply to the blade is cut if its operating temperature exceeds 350°C (7).

Because the THS heats the tobacco, instead of burning it, THS emits significantly reduced levels of toxicants than cigarettes. The harmful and potentially harmful constituents (HPHC) measured in THS aerosol, including carcinogens, cardiovascular, reproductive, developmental, and respiratory toxicants (based on a list published by the FDA in 2012; ref. 6), are reduced by more than 90% on average in both the regular and menthol THS variants, compared with levels found in cigarette smoke (8–10). This reduction in emissions led to a (90% or greater) reduction in both *in vitro* cytotoxicity and mutagenicity of both gas and particulate phases of the THS aerosol compared with the smoke of the 3R4F reference cigarette (11).

In a series of randomized clinical trials, it has been shown that biomarkers of exposure (BoExp) to HPHCs are reduced substantially in smokers switching completely to THS after only 5 days. These changes were largely maintained in an ambulatory setting, over a period of 3 months, in Japanese and American smokers who switched to THS (12, 13). In addition to favorable changes in BoExp, changes in biomarkers of effect have been shown to approach those seen in smokers who quit using tobacco products altogether (13, 14).

In this study, aside from replication of previous findings aimed at confirmatory testing of prespecified research hypotheses, favorable changes in a set of 8 biomarkers of effect tested as co-primary endpoints (HDL-C, WBC, FEV_1%pred, COHb, Total NNAL, sICAM-1, 11-DTX-B2, 8-epi-PGF2α) were to be confirmed in a larger sample of U.S. smokers over an extended period of 6 months after switching from cigarettes to THS. In addition, biomarkers of exposure were assessed as secondary endpoints (MHBMA, 3-HPMA, Total NNN, CEMA, 3-OH-B[a]P, HMPMA, Total 1-OHP, NEQ, CO exhaled).

Materials and Methods

Study design

The study was a randomized, controlled, 2-arm parallel group, multicenter, open-label, ambulatory trial to evaluate biological and functional changes in healthy smokers who switched to THS use compared with those who continued to smoke conventional cigarettes for 26 weeks in an ambulatory setting. It was conducted at 20 study sites, clinical trial facilities managed by independent contract research organizations (CRO), with various experiences in phase I, phase II, and phase III trials, located in Arizona, Florida, Kentucky, Nebraska, Nevada, North Carolina, Ohio, Tennessee, Texas, and Virginia. The Midlands Independent Review Board (IRB) approved the study, and all participants provided written informed consent prior to enrollment. Recruitment started in March 2015, and the last subject completed the study in September 2016. Participants were randomized to either THS or continued cigarette smoking. Stratified randomization ensured that each sex represented at least 40% of the study population at each site and in each arm. A quota was applied to ensure that participants of European origin did not represent more than 75% of the randomized participants. The protocol was shared with the FDA prior to study conduct and the study design was published on ClinicalTrials.gov (NCT02396381; https://www.clinicaltrials.gov/ct2/show/NCT02396381). As described in the protocol, no Data Safety and Monitoring Board was employed for this study. Reporting of SAEs was outsourced to United Biosource Corporation and AEs were handled by Covance Medical Monitoring.

Participants

Participants were recruited directly by each clinical study site, from their existing database of study volunteers and via local advertising, after each study site received IRB approval. The study adhered to the principles of Good Clinical Practice, including the requirements for obtaining informed consent. Study participants were notified that they were free to withdraw their participation at any time. Compensation was provided to participants, as per IRB approval and according to a predefined payment schedule, irrespective of their actual tobacco product use.

Participants were at least 30 years old, healthy, male or female smokers (verified by a urinary cotinine test $\geq$200 ng/mL) not motivated to quit within the next 6 months. They had smoked at least 10 commercially available nonmenthol cigarettes per day for the last year and had been smoking for at least 10 years. Major exclusion criteria included $FEV_1/FVC < 0.7$ and $FEV_1 < 80\%$ predicted value at post-bronchodilator spirometry, asthma (post-bronchodilator $FEV_1/FVC < 0.75$ and reversibility in FEV_1%pred $\geq$12% and >200 mL from pre- to post-bronchodilator values), and a body mass index (BMI) <18.5 or $\geq$35 kg/m^2. Pregnant or breastfeeding women were not eligible for participation. Each participant, who met the study eligibility criteria, received a demonstration of THS upon their first visit to the study site.

Procedures

Eligible subjects received containers for home collection of 24-hour urine, which they subsequently checked in for enrollment and baseline assessments. All participants received training on how to use THS, before using it *ad libitum* during an ambulatory run-in period of 8 ± 2 days, and were to record all use of tobacco/nicotine-containing products with an electronic diary. The run-in

was implemented for the subjects to get familiarized with the product use (handling and taste) before randomization. On the randomization day, subjects returned the THS set, including the unused *HeatSticks*, back to the site staff. After randomization, subjects either continued smoking their own brand of cigarettes or were dispensed THS, if allocated to the THS group. Participants were asked to self-report the number of cigarettes and *HeatSticks* used daily in an electronic diary. Subjects allocated to the THS arm were instructed to use the product exclusively during the 6-month period at their discretion. Any subject who wanted to quit THS was encouraged to do so but was asked to attend all further scheduled visits for assessments.

During the postrandomization period, 6 monthly safety checks took place, and participants were resupplied with *HeatSticks* to cover their needs until the next visit, based on their recorded consumption during the preceding period. At the Month 3 and Month 6 visits, blood and 24-hour urine were collected for assessments of BoExp and primary endpoints. Urine collection started at home in the mornings of the days before these visits. The end of the study was the checkout of the Month 6 visit or the date of early termination, plus a 28-day safety follow-up period.

Rationale for biomarker selection

The BoExp measured in this study cover HPHCs of both the gas and particulate phases of cigarette smoke and are representative of a variety of chemical and toxicological classes (carcinogens, reproductive or developmental, cardiovascular, and respiratory toxicants). They also reflect the adverse health effects of HPHCs that affect multiple organ systems, disease pathways, and mechanisms (15, 16). Each primary endpoint was identified through a review of the existing science (17, 18).

The published clinical and epidemiological research was assessed systematically to confirm (i) a robust relationship between each primary endpoint and at least one known smoking-related disease, (ii) clinical evidence linking cigarette smoking to negative changes in each primary endpoint, and (iii) reversibility (favorable changes) of each primary endpoint within 6 to 12 months following smoking cessation.

No single biomarker can fully capture a clinical endpoint. A single biomarker of effect could predict several clinical conditions, so there could be biomarkers of systemic inflammation or oxidative stress relating to cardiovascular disease, cancer, and lung disease. Other intermediate endpoints could be more specific to certain conditions, such as FEV_1%pred for lung function. For this reason, more than one biomarker of effect was included in the assessment of the effects of switching from cigarettes to THS.

Outcomes

The primary outcome was the change in a set of 8 co-primary endpoints (Supplementary Table S1) at Month 6 in smokers switching from conventional cigarettes to THS as compared with those continuing to smoke cigarettes. The 8 endpoints were high-density lipoprotein cholesterol (HDL-C) in serum; white blood cell (WBC) total count in blood; soluble intercellular adhesion molecule-1 (sICAM-1) in serum; carboxyhemoglobin (COHb) in blood; forced expiratory volume in one second (FEV_1) post-bronchodilator, expressed as % predicted (FEV_1%pred); and the following measured in urine and adjusted for creatinine: 11 dehydrothromboxane B2 (11-DTX-B2), 8-epi-prostaglandin F2 alpha (8-epi-$PGF_{2\alpha}$), total 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (Total NNAL). Total NNAL, while a biomarker of exposure to NNK, was a primary endpoint of the present study because of its reported potential to cause cancer-initiating DNA damage (19).

Secondary outcomes included the following BoExp: carbon monoxide (CO) in exhaled breath, monohydroxybutenyl-mercapturic acid (MHBMA), 3-hydroxypropylmercapturic acid (3-HPMA), total N-nitrosonornicotine (Total NNN), 2-cyanoethylmercapturic acid (CEMA), 3-hydroxybenzo(a)pyrene (3-OH-B[a]P), 3-hydroxy-1-methylpropylmercapturic acid (3-HMPMA), and total 1-hydroxypyrene (Total 1-OHP). To describe nicotine exposure, in addition to plasma levels of nicotine and cotinine, nicotine equivalents (Neq) were determined as the molar sum of free nicotine, nicotine-glucuronide, free cotinine, cotinine-glucuronide, free trans-3′-hydroxycotinine, and trans-3′-hydroxycotinine-glucuronide in urine (expressed as concentration adjusted for creatinine).

The bioanalytical methods used were all validated either according to the College of American Pathologists/Clinical Laboratory Improvement Amendments CAP/CLIA regulations or the Bioanalytical Method Validation guidance for Industry, from Food Drug Administration (2013) with preestablished intra- and inter-precision assay (20). HDL-C, WBC, and sICAM-1 were measured by Covance Central Laboratories Services, Inc., Indianapolis. MHBMA, 3-HPMA, Total NNAL, COHb, 11-DTX-B2, 8-epi-PGF2α, Total NNN, CEMA, 3-OH-B[a]P, 3-HMPMA, Total 1-OHP, and Neq were analyzed at Celerion Laboratories (Supplementary Table S1). Biomarker assay analyses were not batched, but were performed on an ongoing basis and blinded with respect to randomization. A description of the bioanalytical methods used to measure creatinine and BoExp was published previously (21).

Additional secondary endpoints, such as cough symptoms, product evaluation [satisfaction, reward, aversion, enjoyment, and craving reduction 7-point Likert subscale averages from the modified Cigarette Evaluation Questionnaire (mCEQ; ref. 22), the Fagerström Test for Nicotine Dependence (FTND; ref. 23)], and intention to use THS in the future were recorded.

Adverse events (AE), serious adverse events (SAE), device events, vital signs, body weight, and BMI were collected for the duration of the study.

Statistical analysis

To reflect naturalistic use of THS in the first months after switching, full analysis set exposure groups were defined *a priori* based on actual tobacco product use categories for the 6-month postrandomization period. The full analysis set consisted of all randomized participants with recorded postrandomization product use, with a baseline and at least one postrandomization value for one of the primary endpoints. The predominant THS use category was defined as $\geq$70% THS use on average over the 6-month period and, in addition, on at least half of the days of the overall period. Predominant cigarette smoking was defined as <1% THS use and on at least half of the days of the overall period. Dual use was defined as 1% to <70% THS use.

Assuming that 75% of the participants randomized to THS would fulfill the criteria for predominant THS use after randomization, the sample size was calculated to ensure an overall study power of at least 90% while maintaining at least 80% power to detect the expected effect of THS use as compared with continued cigarette smoking for each co-primary endpoint (Supplementary Table S2). For FEV_1%pred, the test-wise power was calculated to

be 82.2% and was higher for all other co-primary endpoints, using the Hailperin–Rüger approach (24, 25), with a 2-sided type I error probability of 0.03125 for each co-primary endpoint while preserving the overall 5% family-wise error rate. A sample size of 475 participants in both the THS and continued cigarette smoking groups provided more than 99% power to demonstrate at least 5 of 8 statistically significant favorable changes in the co-primary endpoints.

The 8 co-primary endpoints were analyzed at Month 6 for the comparison between predominant THS use and cigarette smoking, either after natural log-transformation (sICAM-1, 11-DTX-B2, 8-epi-$PGF_{2\alpha}$, COHb, and Total NNAL) or in the original scale (HDL-C, WBC, and FEV_1%pred), with urinary endpoint concentrations being adjusted for creatinine. A mixed-effect analysis model for repeated measurements including participants with complete data for all model terms was used for each co-primary endpoint, adjusted for the fixed effects of sex, ethnicity (European vs. other), time point (Months 3 and 6), baseline endpoint value and its interaction with time point, product use group and its interaction with time point, other baseline covariates relevant for each specific endpoint, and site as a random effect. The same analysis approach was used to describe the adjusted mCEQ subscale mean differences between predominant THS use and cigarette smoking at Month 6. To explore the effects of switching to THS on the need to cough (Yes/No), a logistic regression model was used based on the same linear predictor, but time point was treated as a random effect, and age and smoking intensity were included as additional covariates. Data missing at baseline and after randomization were considered as missing completely at random and at random, respectively.

To protect the study-wise α-level of 5%, the Hailperin–Rüger method was used to determine a test-wise, one-sided type I error level of 1.5625% that is required for each of the 8 tests of the co-primary endpoints, when at least 5 of them are required to be significantly changed in the expected direction, without the need for prior specification as to which of the 8 endpoints are required to reach statistical significance. Model-based least square (LS) means and THS minus cigarette smoking difference estimates at Month 6 were calculated for HDL-C, WBC, and FEV_1%pred, along with 2-sided 96.875% ($100 - \alpha$%) confidence intervals (CI) and *P* values. For the other primary endpoints, log-scale results were back transformed to the original scale, with the results presenting percent reductions in the predominant THS use group relative to the cigarette smoking group. The safety population consisted of all enrolled participants with at least one measurement for safety assessment.

Exploratory *post hoc* analyses were conducted in the THS group to investigate the magnitude of observed effects in dependency of the degree of exposure from using cigarettes. This analysis was conducted for strata of CEMA quartiles in the THS group. Supplementary Tables S5 to S8 contain the results from an additional analysis comparing subjects as randomized. All analyses were performed with SAS 9.2.

Results

Figure 1 summarizes the flow of participants through the study. In total, 803 (81.6%) of the 984 randomized participants completed Month 6. One site was terminated due to noncompliance with Good Clinical Practice guidelines, as identified during a for-cause audit. The data collected from this site were included in the full safety population ($n = 1{,}039$) only.

Of the 488 participants randomized to THS, 245 were in the postrandomization predominant THS use category, 142 were in the dual use category, and 3 were in the cigarette smoking category (because they were never exposed to THS during the study), based on self-reported product use. Of the 428 participants in the postrandomization cigarette smoking category, 425 had been randomized to cigarette smoking and 3 participants had been randomized to THS but had continued to smoke cigarettes (Fig. 1). The baseline characteristics of the participants of the 3 product use category groups (predominant THS use, dual use, cigarette smoking), including smoking history and characteristics, are shown in Table 1. The 815 participants were, on average, between 44 and 45 years of age; 476 (59%) were male, and 649 (80%) were white, of whom 606 (74%) self-reported their ethnicity as not hispanic or latino.

Respiratory function was normal in more than 90% of the participants in either product use category group. The participants' baseline smoking intensity was, on average, at about 19 cigarettes per day, and they had smoked, on average, for about 25 to 27 years. According to the FTND, between 43% and 47% were categorized as moderately nicotine dependent (4 to 6 points), and between 39% and 40% were categorized as severely nicotine dependent.

Postrandomization, the 428 cigarette group participants smoked on average 16.8±6.8 cigarettes per day. In the dual-use group, the average daily consumption of THS was 7.6±5.2 and that of cigarettes was 10.0±5.9. In the THS use group, the average daily consumption of THS was 16.5±8.9 and that of cigarettes was 2.0±2.4.

Table 2 contains the 6-month mixed-effect model-based findings on the set of the 8 co-primary endpoints. Five of the 8 co-primary endpoints showed statistically significant favorable differences between subjects in the predominant THS use group as compared with the continued cigarette smoking group at Month 6, using a one-sided test with the Hailperin–Rüger adjusted α level of 1.5625%, following the predefined hypothesis testing procedure. Although somewhat smaller in general, the magnitude of the observed effect was close to the expectation for HDL-C, WBC count, FEV_1%pred, and Total NNAL, but only about half as large as expected for 8-epi-$PGF_{2\alpha}$ and COHb, and even smaller for sICAM-1 and 11-DTX-B2, which like 8-epi-$PGF_{2\alpha}$ showed favorable but nonsignificant shifts in the expected direction.

The effect magnitudes were further explored by reestimating the model-based Month 6 effects separately for each of the 4 quartiles of the CEMA distribution in the predominant THS group at Month 6. Acrylonitrile is generated at temperatures ranging from 500°C to 800°C (5) and is reduced by over 99% in the THS aerosol (11). Therefore, CEMA, the biomarker of exposure for acrylonitrile exposure, can be used as an objective marker to distinguish cigarette smoking from THS use. The upper bounds of the CEMA quartiles and, in parentheses, the size of the quartile and the mean (±SD) self-reported number of cigarettes smoked per day (cpd), were 32 ng/mg creat ($n = 56$; 0.6 ± 1.2 cpd), 128 ng/mg creat ($n = 56$; 1.6 ± 1.9 cpd), 227 ng/mg creat ($n = 57$; 2.1 ± 2.5 cpd), and 784 ng/mg creat ($n = 56$; 2.3 ± 2.8 cpd). The results for the co-primary endpoints by CEMA quartile are shown in Fig. 2.

Monotonous trends of more pronounced effects from higher to lower CEMA quartiles were visible for 8-epi-$PGF_{2\alpha}$, COHb,

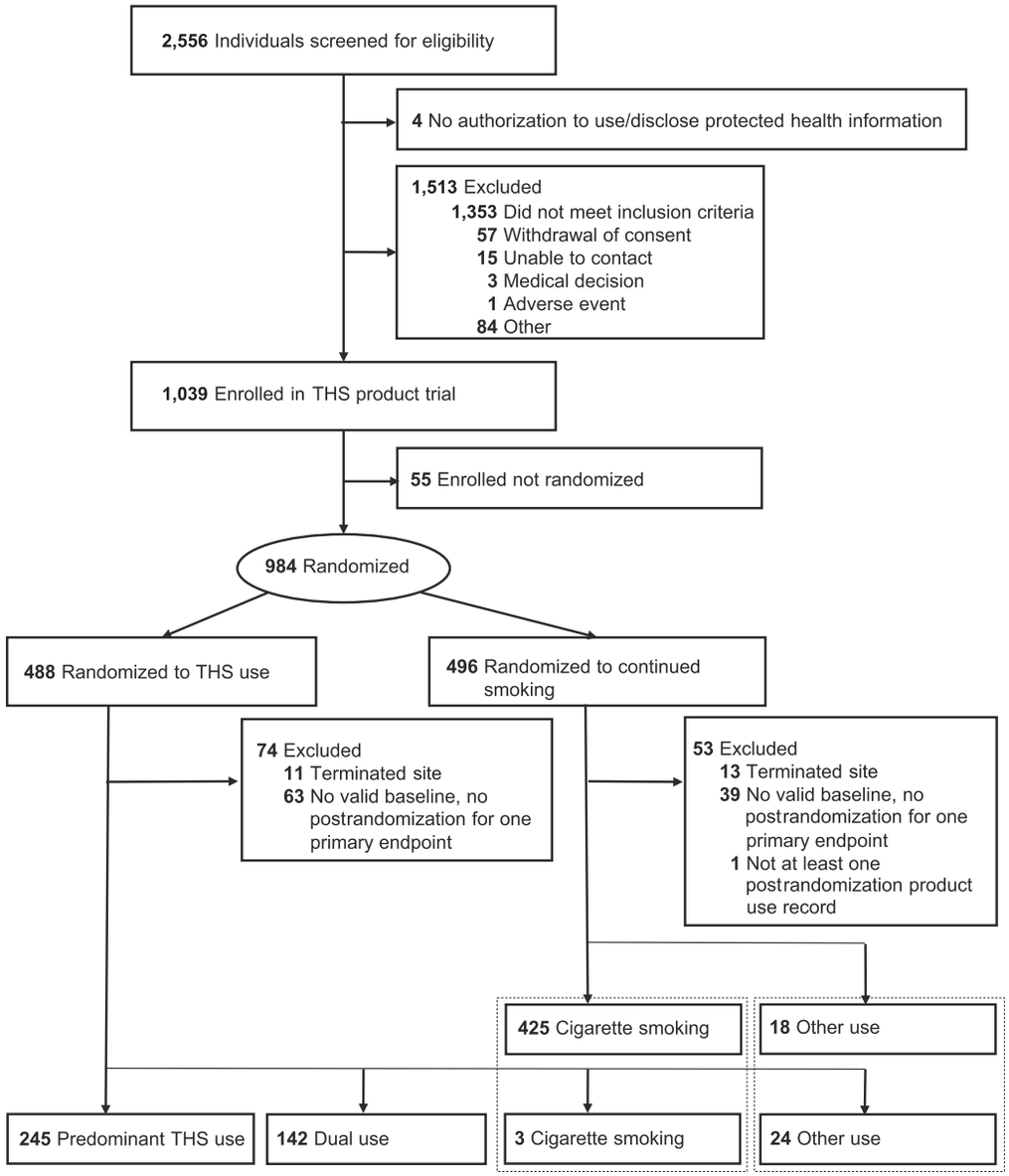

Figure 1.

Flow of participants through the study. Note: "Other use" is a general category encompassing subjects with missing product use, subjects using prevalently e-cigarettes or other tobacco products, subjects who quit, or subjects who switched across different use patterns between consecutive analysis periods. Among the 42 subjects in other use, 21 had missing product use category, 6 subjects were primarily e-cigarette users, 3 subjects primarily other tobacco users, and 12 subjects switching across categories between Months 1 to 3 and Months 4 to 6.

sICAM-1, Total NNAL, FEV_1%pred, and WBC counts. The 95% CIs of the effect estimates in the lowest (first) CEMA quartiles did not cover the no-effect thresholds of zero or unity for THS versus cigarette smoking group differences or ratios, respectively, with the only exception being 11-DTX-B2. In the first CEMA quartiles, the observed reduction effects from smoking were quite close to those expected (Supplementary Table S3) for HDL-C [2.8 mg/dL (95% CI, 0.1–5.6)], sICAM-1 [9% (95% CI, 4–14)], and 11-DTX-B2 [11.3% (95% CI, −8.0 to 27.2)] or even larger than expected for WBC count [−0.93 GI/L (95% CI, −1.38 to −0.49)], FEV_1 [2.3% pred. (95% CI, 0.4–4.2)], 8-epi-$PGF_{2\alpha}$ [26.2% (95% CI, 17.3–34.1)], COHb [70% (95% CI, 65–74)], and Total NNAL [89.0% (95% CI, 86.1–91.3)].

The LS means percent changes in BoExp at Month 6 are shown in Fig. 3 for the THS and dual use groups relative to the continued cigarette smoking group. Supplementary Table S4 provides the accompanying numerical values and abbreviations.

All BoExp were markedly reduced in the predominant THS use group compared with the cigarette smoking group, whereas for Neq, no difference was seen between the groups. A markedly lower reduction in BoExp levels was observed in dual users (Fig. 3).

Table 1. Summary of demographic data and smoking-related characteristics at baseline; full analysis set by product use group

	Product use group		
Variable	**THS (*N* = 245)**	**Dual (*N* = 142)**	**Cigarettes (*N* = 428)**
Sex[a]			
Male	151 (61.6)	79 (55.6)	246 (57.5)
Female	94 (38.4)	63 (44.4)	182 (42.5)
Age (years)[b]	44.2 (9.64)	43.8 (9.77)	45.2 (9.55)
Race[a]			
White	195 (79.6)	113 (79.6)	341 (79.7)
African American	42 (17.1)	25 (17.6)	74 (17.3)
Other	8 (3.3)	4 (2.8)	13 (3.0)
Educational attainment[a]			
Less than high school	11 (4.5)	9 (6.3)	24 (5.6)
High school	145 (59.2)	93 (65.5)	270 (63.1)
College and higher	89 (36.3)	39 (27.5)	133 (31.1)
Height (cm)[b]	173 (10.5)	173 (8.7)	173 (9.6)
Weight (kg)[b]	80.9 (16.8)	80.4 (15.4)	81.0 (15.6)
Underweight[a]	1 (0.4)	0	0
Normal weight[a]	82 (33.5)	51 (35.9)	144 (33.6)
Overweight[a]	96 (39.2)	52 (36.6)	165 (38.6)
Obese[a]	66 (26.9)	39 (27.5)	119 (27.8)
Body mass index (kg/m^2)[b]	26.9 (3.99)	26.9 (4.35)	27.1 (4.13)
COPD staging[a]			
Normal	224 (91.4)	131 (92.3)	400 (93.5)
GOLD1: Mild	18 (7.3)	7 (4.9)	24 (5.6)
GOLD2: Moderate	3 (1.2)	4 (2.8)	4 (0.9)
Smoking duration (years)[b]	25.7 (9.5)	25.5 (10.0)	26.7 (10.1)
Pack years[b]	22.8 (12.5)	23.4 (14.2)	25.0 (15.8)
Cigarettes per day over the last year[b]	18.5 (7.1)	19.5 (7.7)	19.5 (7.9)
Fagerström test for nicotine dependence[b]	5.7 (2.1)	5.7 (2.2)	5.8 (2.0)
Mild[a]	39 (15.9)	26 (18.3)	57 (13.3)
Moderate[a]	107 (43.7)	61 (43.0)	199 (46.5)
Severe[a]	97 (39.6)	55 (38.7)	167 (39.0)
Nicotine equivalent (mg/g creat)[c]	9.2 (88.7)	10.3 (70.4)	10.1 (78.9)

[a]*n* (%).
[b]Mean (SD).
[c]Mean (coefficient of variation, %) of creatinine-adjusted molar sum of free nicotine, nicotine-glucuronide, free cotinine, cotinine-glucuronide, free trans-3′-hydroxycotinine, trans-3′-hydroxycotinine-glucuronide in urine.

Baseline Neq levels ranged between 9.2 mg/g creatinine and 10.3 mg/g creatinine across the 3 product use category groups. At Month 6, the geometric LS means were almost identical in the predominant THS use and cigarette smoking groups at 8.9 and 8.9 mg/g creat., respectively. The predominant THS use versus cigarette smoking LS means ratio was 101% with 95% CI values ranging from 91.2% to 111%. In the dual use group, the 6-month LS Neq mean was slightly lower at 8.3 mg/g creat., and the dual use over cigarettes LS means ratio was 94.1% (95% CI, 83.6–106).

Table 2. THS versus cigarette smoking group effects in the primary endpoints

Endpoint	**Effect[a]**	**96.875% CI**		***P* value[b]**
HDL-C (mg/dL)[c]	3.09	1.10	5.09	<0.001*
WBC count (GI/L)[c]	-0.420	-0.717	-0.123	0.001*
sICAM-1 (%)[d]	2.86	-0.426	6.04	0.030
11-DTX-B2 (%)[d]	4.74	-7.50	15.6	0.193
8-epi-PGF$_{2\alpha}$ (%)[d]	6.8	-0.216	13.3	0.018
COHb (%)[d]	32.2	24.5	39.0	<0.001*
FEV1%pred (post-bronchodil.)[c]	1.28	0.145	2.42	0.008*
Total NNAL (%)[d]	43.5	33.7	51.9	<0.001*

*Significant at the 1.5625% type I error level.
[a]LS Mean difference or relative change.
[b]One-sided.
[c]Difference.
[d]%Reduction.

The mCEQ questionnaire included instructions to subjects to evaluate the product they were allocated to. Therefore, THS participants in the THS arm evaluated how using THS made them feel and subjects in the cigarette arm evaluated their own cigarettes. The 5 mCEQ subscales Month 6 LS means were very similar in predominant THS users versus cigarette smokers for satisfaction (4.5 vs. 4.6), reward (4.2 vs. 4.3), aversion (1.4 vs. 1.5), enjoyment (3.9 in both groups), and craving reduction (5.0 in both groups), with the mean difference 95% CI covering zero in each instance.

At Month 6, in response to an Intent to Use questionnaire, 58% of the predominant THS use group participants and 30% of the dual use group participants stated that they would "very likely" or "definitely" use THS regularly. Concerning intentions for dual use, 19% of the predominant THS use group and 46% of the dual use group stated that they would consume both THS and cigarettes. At baseline, in subsequent THS and dual users, the proportions of THS use intentions had been 64% and 42%, respectively.

No SAEs related to either THS use or cigarette smoking were reported by any randomized subject, and no participants were discontinued due to product-related AEs. Of the predominant THS users, 197 reported 358 AEs, compared with 218 smokers who reported 400 AEs, with a total of 19 AEs classified as severe by the investigators. The incidence of product use-related AEs, most frequently cough, was higher in the THS group (11 AEs in 8 participants, of which 2 reported cough) compared with the dual use (3 AEs in 3 participants, of which 2 reported cough) and continued smoking groups (2 AEs in 2 participants, of which 1 reported cough).

At Month 6, the model-based prevalence of participants with a self-reported regular need to cough was 20.8% in predominant THS users compared with 30.7% in continued smokers (OR = 0.6; 95% CI, 0.4–0.9), whereas in the dual use group, the prevalence of 28.7% was similar to that in continued smokers (OR = 0.9; 95% CI, 0.6–1.5).

Discussion

This study aimed at providing confirmatory evidence that switching from cigarettes predominantly or completely to THS leads to favorable changes in primary endpoints considered to be relevant for smoking-related diseases.

About half of the smokers allocated to THS actually used the product predominantly (as defined above) over the 6-month product use period. Nearly one-third of the THS arm substituted cigarettes partially by engaging in dual use and cutting down their baseline cigarette consumption by an average of nearly one-half in the process, although CEMA levels indicate that self-reported use overestimated THS use over cigarette smoking.

After switching to predominant THS use the subjective effects, as self-reported with the mCEQ, approached those of smokers. The product was found to be acceptable by a majority of the predominant THS users, which is in line with the acceptance of the product in countries where it is already marketed; in Japan, after about 3 years some 3.8 million smokers have adopted THS, almost 9 of 10 using heated tobacco exclusively (26). Specifically, at Month 6, no notable difference was detectable between predominant THS users and cigarette smokers regarding craving

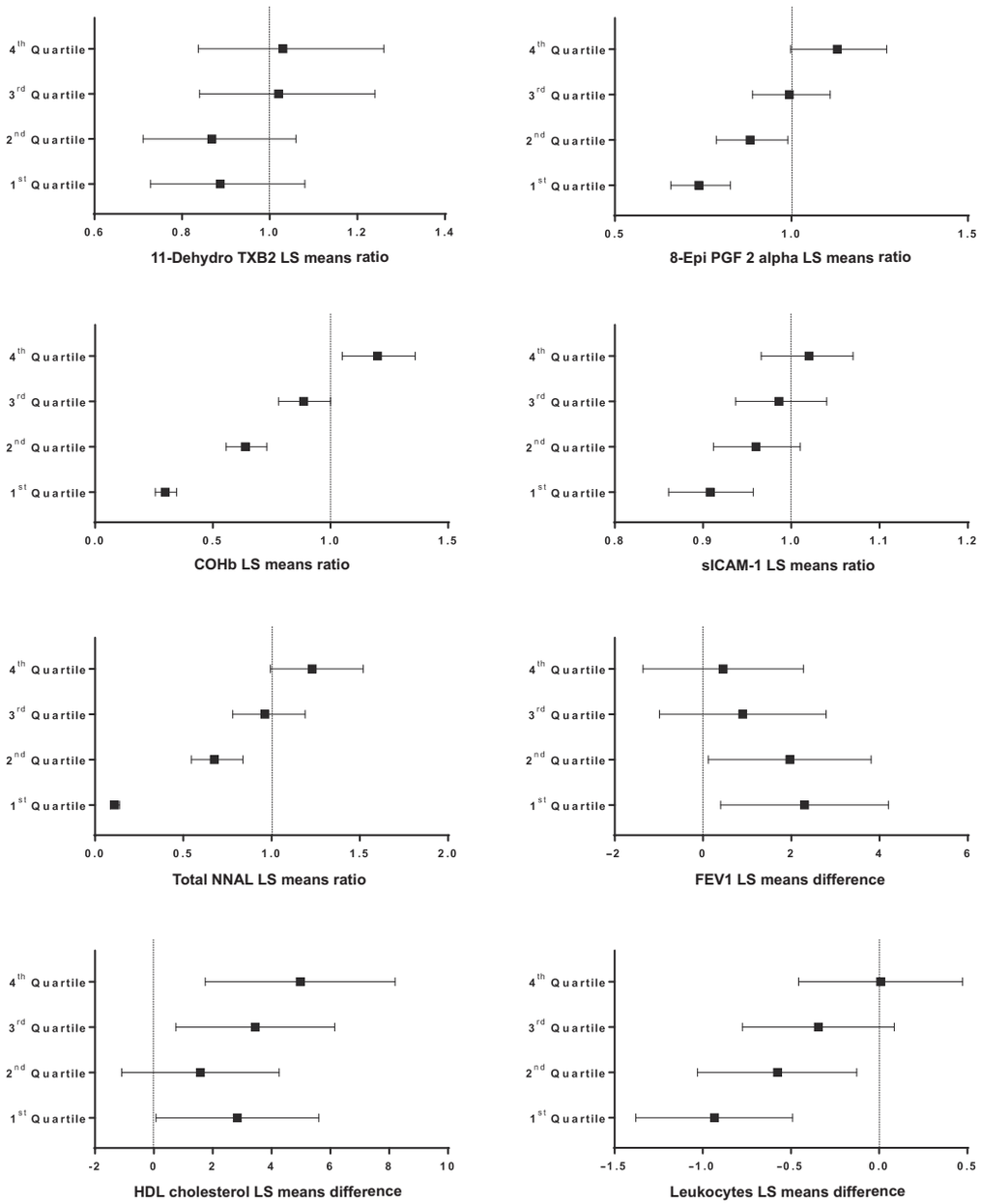

Figure 2.

Model-based Month 6 effect estimates and 95% confidence intervals in the 8 co-primary endpoints. The predominant THS use category group was stratified by CEMA quartiles 1 (bottom) to 4 (top). Higher CEMA levels are indicative of higher levels of cigarette smoking. The top 5 panels show the predominant THS versus continued cigarette smoking LS means ratios, and the last 3 panels show the FEV1%pred, HDL-C, and leukocytes predominant THS minus cigarette smoking LS means differences.

reduction, psychological reward, smoking satisfaction, aversion, and enjoyment of respiratory tract sensation. The finding is braced by the equal nicotine levels in predominant THS users and cigarette smokers at the end of the study. In line with these findings, nearly 2 of 3 predominant THS users and almost every third dual user intended to use the product in the future. Approximately half of the predominant THS users self-reported their intent to continue to use THS regularly as "very likely" or "definitely." These results are, however, not necessarily predictive of future product acceptance by smokers because THS was not available in the U.S. market and the study participants may not have seriously considered the option.

The study met the criteria of collectively improved co-primary endpoints (biomarkers of effect), with all of them shifting in the same direction as with smoking cessation. The set of primary endpoints covered multiple biological mechanisms that are involved in the development of smoking-related diseases over years. Compared with continued cigarette smoking, switching to

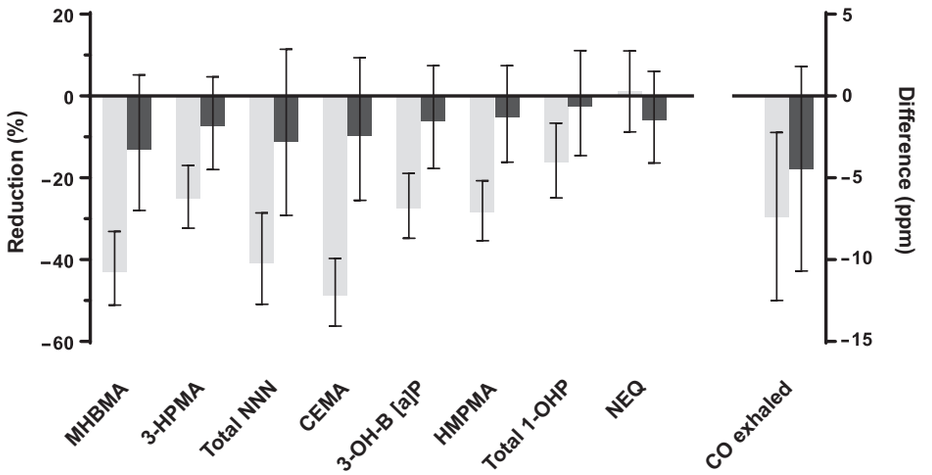

Figure 3.
THS:cigarettes (light gray) and dual use:cigarettes (dark gray) percent reductions and CO exhaled difference between THS and dual use versus cigarettes, and 95% confidence intervals at Month 6. Supplementary Table S4 displays the accompanying numerical values and abbreviations.

THS resulted in HDL-C increase (P-value <0.001), reduced WBC counts (P-value = 0.001), higher FEV1%pred (P-value = 0.008), decreased COHb (P-value <0.001), and reduced exposure to carcinogens (Total NNAL; P-value < 0.001). Favorable changes were also observed in platelet activation (11-DTX-B2; P-value = 0.193), endothelial dysfunction (sICAM-1; P-value = 0.030), and in oxidative stress (8-epi-PGF2α; P-value = 0.018). The family-wise type I error rate was strictly controlled at a one-sided α-level of 1.5625% with the use of the Hailperin–Rüger approach (24, 25).

Although exposure reductions were also seen in the dual use group, the effects were, in line with expectations based on dose–response considerations, approximately twice as large, but mostly larger, in the predominant THS user group, where they ranged from 16% to 49%. Previous findings regarding the reductions of the same BoExp in the 3-month ambulatory study conducted in the United States were larger and reported between 33% and 86% (13). The most likely causes for these differences are the design and procedures of this study, where (i) urine samples were self-collected at home, whereas in previous studies urine samples were collected on site during a 2-day visit, (ii) the categorization of the predominant THS use group being based on self-reported product use, and (iii) allowing up to 30% concomitant smoking in the predominant THS use group.

The BoExp- and primary endpoint-related results of the study need to be interpreted in the context of product use patterns, as, on average, study participants in the predominant THS use category also smoked some cigarettes. Therefore, the results reflect the changes that can occur under real-life conditions during the adoption and conversion process in adult smokers who reduce their levels of exposure to HPHCs by switching to THS.

To gain further insights into the dose–response relationships between concomitant product use and the changes in co-primary endpoints, a *post hoc* analysis was conducted. This analysis was based on the objective combustion biomarker CEMA. In this analysis, the effects of the concomitant cigarette smoke exposure on each of the 8 co-primary endpoints were analyzed by stratifying the study participants in the THS use group by the 6-month CEMA concentration quartiles.

In particular, in the first quartile, with an upper CEMA concentration of 32 ng/mg creat. (and 0.6 cigarettes smoked per day on average), additional smoking was low. For 7 of the 8 co-primary endpoints, the effect estimates were clearly most pronounced in the first and second quartiles compared with the third and fourth quartiles. In the lowest 2 quartiles of these endpoints, the effect sizes approached those expected when approximately 60% to 80% of the effects of smoking cessation are retained.

As with the BoExp, the dose–response seen in the primary endpoints in the present study, in particular when exposure reduction is further improved by predominant THS users abstaining from cigarette smoking, are consistent with effects seen in smoking cessation (17, 18, 27) but also in smokers who reduced their daily cigarette consumption (28–30).

THS and cigarettes were equally well tolerated throughout the study, and there was no discontinuation due to product-related AEs; the proportion of subjects reporting a regular need to cough was lower in predominant THS users compared with smokers. Together with the use prevalence, subjective effects, nicotine levels, and intentions to use the product in the future, this indicates that THS is an acceptable alternative to smoking, therefore providing a suitable substitute for cigarettes to adult smokers who would otherwise continue smoking.

In summary, the results of this study point toward THS being an acceptable alternative for smokers, as well as toward THS being a product that implicates substantially reduced exposure to HPHCs, as demonstrated by the reductions over a large range of BoExp. Together with the finding that primary endpoints were improved significantly, it can be inferred that THS is a modified risk tobacco product if there is predominant substitution of cigarettes. It should be pointed out that this inference on long-term harm reduction is, necessarily, based on evidence regarding short-term endpoints. However, as pointed out in a recent policy statement of the American Thoracic Society on tobacco harm reduction claims, such inference is endorsed by them acknowledging that "Inference is an acceptable technique for estimating the anticipated impact of harm reduction strategies. . ." (31).

There are 2 caveats: the first relating to the perception of the messenger, the second to the disease prevention potential-related implications of the findings.

With regard to the first caveat, because of skepticism associated with studies conducted by tobacco industry funded research and scientists, replication by independent researchers would further support our results. It should be noted that if our findings are indeed accurate, they are of great importance to individual

smokers as well as public health, as they point toward a feasible route for harm reduction by potentially reducing the burden of smoking-related diseases in smokers who would otherwise continue smoking.

This brings us to the second caveat, which expands upon the likelihood of smokers switching to THS and thereby reducing their individual risks of harm and smoking-related diseases. As subjects did not have to pay for THS in this study, the observed levels of substitution and use may not generalize to the real world. In this context, it is important to note that the net public health benefit of the market introduction of an MRTP is a function of both the individual risk reduction potential of the introduced MRTP and of the distribution of tobacco product consumption patterns in the population over time (32).

Based on the findings of this study, not all participants in the THS arm switched predominantly to THS after 6 months. It has to be considered, however, that randomization does not accurately reflect real-life self-selection of a consumer product. Also, the study duration of 6 months and the lack of public availability and general use of THS might restrict more comprehensive behavioral transition from smoking to exclusive THS use.

MRTPs should not attract individuals who do not currently use tobacco products, that is never smokers or former smokers, and should not negatively influence smokers who intend to quit. Although it is possible to address this issue to some degree prior to market introduction with population health impact modeling (32–34), postmarket surveillance programs and studies are needed to monitor the epidemiological development in the real world.

Such postmarket surveillance is also important for monitoring real-world transition from cigarette smoking to THS use, in particular exclusive THS use (26).

In conclusion, all co-primary endpoints showed favorable changes in the same direction as with smoking cessation and improved biological effects were observed primarily in the smokers who predominantly used THS compared with continued cigarette smoking, with nicotine levels and subjective effects being similar in both groups. The improvements were statistically significant in 5 of the 8 co-primary endpoints at Month 6, confirming the hypothesis that THS is an acceptable alternative to cigarettes for smokers and, based on the positive biological effects, likely presents less risk of harm than continued smoking.

Disclosure of Potential Conflicts of Interest

S.M. Ansari is a Clinical Scientist at Philip Morris International. N. Lama is a Senior Scientist – Statistics at Philip Morris International. P. Picavet has ownership interest (including stock, patents, etc.) in Philip Morris International. G. Baker has ownership interest (including stock, patents, etc.) in Philip Morris International. M. Peitsch has ownership interest (including stock, patents, etc.) in Philip Morris International. R. Weitkunat has ownership interest (including stock, patents, etc.) in Philip Morris International. No potential conflicts of interest were disclosed by the other authors.

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Study supervision: F. Lüdicke, G. Baker

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