PMI RESEARCH & DEVELOPMENT

Clinical Study Protocol

Study title:	A cross-sectional, multi-regional study to demonstrate reduction in exposure to key toxicants, oxidative stress, and inflammation following at least 2 years of Tobacco Heating System (THS) use compared to cigarette smoking.
Study number	P1-RMC-03-INT
Short title	Reduction of exposure, inflammation, and oxidative stress following at least 2 years of switching to THS use compared to cigarette smoking.
Product name:	Tobacco Heating System (THS)
Sponsor:	Philip Morris Products S.A. Quai Jeanrenaud 5 2000 Neuchâtel Switzerland
Version:	3.0, Approved
Date:	07 Jan 2022

SYNOPSIS

Sponsor:

Philip Morris Products S.A.
Quai Jeanrenaud 5
2000 Neuchâtel
Switzerland

Name of Product:

Tobacco Heating System (THS)

Study Title:

A cross-sectional, multi-regional study to demonstrate reduction in exposure to key toxicants, oxidative stress, and inflammation following at least 2 years of Tobacco Heating System (THS) use compared to cigarette smoking.

Study Number:

P1-RMC-03-INT

Short Title:

Reduction in exposure, inflammation, and oxidative stress following at least 2 years of switching to THS use compared to cigarette smoking.

Primary Objective and Endpoints:

The primary objective of this study is:

1. To demonstrate beneficial differences in exposure to Harmful and Potentially Harmful Constituents (HPHCs), inflammation, and oxidative stress in THS users compared with current cigarette smokers.

   Endpoints:

   - Biomarkers of Exposure (BoExp) to HPHCs:
     - Carboxyhemoglobin (COHb) in blood.
     - Total 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (total NNAL) in urine (expressed as concentration adjusted to creatinine),
   - Biomarkers of Potential Harm (BoPH) for core pathophysiological pathways underlying the main smoking-related diseases:

- Inflammation: White Blood Cell total count (WBC) in blood,
- Oxidative stress: 8-epi-Prostaglandin-$F_{2\alpha}$ (8-epi-$PGF_{2\alpha}$) in urine (expressed as concentration adjusted to creatinine).

Secondary Objectives and Endpoints:

The key secondary objectives of this study are:

2. To demonstrate beneficial differences in lipid metabolism, endothelial dysfunction, or platelet activation between THS users and current cigarette smokers.

   Endpoints:

   - Lipid metabolism: High-Density Lipoprotein Cholesterol (HDL-C),
   - Endothelial dysfunction: soluble Intercellular Adhesion Molecule-1 (sICAM-1)
   - Platelet activation: 11-dehydrothromboxane B2 (11-DTX-B_2,),

3. To demonstrate functional benefits in THS users compared to current cigarette smokers.

   Endpoints:

   - Arterial stiffness: Augmentation Index (AIx),
   - Lung function: Forced Expiratory Volume in 1 second (FEV_1) %predicted, post-bronchodilator (post-BD)

The other secondary objectives of this study are:

4. To determine the differences in BoPH between THS users and former cigarette smokers compared to current cigarette smokers.

   Endpoints:

   - Inflammation:
     - Neutrophil to Lymphocyte Ratio (NLR),
     - High-sensitivity C-Reactive Protein (hs-CRP),
     - Homocysteine (HCY).
   - Oxidative stress:
     - Myeloperoxidase (MPO).
   - Lipid metabolism:
     - Triglycerides (TG).
   - Platelet activation:

- Fibrinogen.

- Glycemic control:
  - Glycated Hemoglobin (HbA1c).
- Lung function (spirometry with and without bronchodilator, absolute and % predicted values, where applicable):
  - FEV_1/FVC.

5. To determine the differences in the levels of BoExp to nicotine between THS users and former cigarette smokers compared to current cigarette smokers.

   Endpoints:

   - BoExp to nicotine:
     - Nicotine Equivalents (NEQ) in urine (expressed as concentration adjusted to creatinine),
     - 2-Cyanoethyl Mercapturic Acid *N*-Acetyl-*S*-(2-cyanoethyl)-L-cysteine (2CyEMA) in urine (expressed as concentrations adjusted to creatinine).

6. To determine the differences in self-reported health perceptions between THS users and former cigarette smokers compared to current cigarette smokers.

   Endpoints:

   - Short Form Health Survey (SF-36) domain scores (Physical Functioning and Mental Health),
   - ABOUT–Health and Functioning domain scores (between THS users and cigarette smokers only).

7. To evaluate the safety during the study.

   Endpoints:

   - Adverse events (AEs),
   - Serious adverse events (SAEs).

Exploratory Objective and Endpoints:

The exploratory objectives of this study are:

- To describe the differences in additional BoPH between THS users and former cigarette smokers compared to current cigarette smokers.

Endpoints:

- Inflammation:
    - WBC (between cigarette smokers and former cigarette smokers only),
    - Interleukin-6 (IL-6),
    - Tumor Necrosis Factor-α (TNF-α),
    - soluble urokinase Plasminogen Activator Receptor (suPAR).
- Oxidative stress:
    - 8-epi-$PGF_{2\alpha}$ (between cigarette smokers and former cigarette smokers only),
    - Malondialdehyde (MDA),
- Lipid metabolism:
    - HDL-C (between cigarette smokers and former cigarette smokers only),
    - Total Cholesterol (TC),
    - Low-Density Lipoprotein Cholesterol (LDL-C),
    - oxidized Low-Density Lipoprotein (ox-LDL),
- Endothelial Dysfunction:
    - sICAM-1 (between cigarette smokers and former cigarette smokers only),
- Platelet Activation:
    - 11-DTX-B_2 (between cigarette smokers and former cigarette smokers only),
- Arterial Stiffness:
    - AIx (between cigarette smokers and former cigarette smokers only),
    - Pulse Wave Velocity (PWV).
- Lung Function (spirometry with and without bronchodilator, absolute and % predicted values, where applicable):
    - Forced Vital Capacity (FVC),
    - FEV_1, (FEV_1 %predicted post-BD between cigarette smokers and former cigarette smokers only)
    - Forced Expiratory Flow between 25% and 75% of FVC ($FEF_{25-75\%}$).
- To describe the differences in the levels of BoExp to HPHCs and nicotine between cigarette smokers and former cigarette smokers.

Endpoints:

- BoExp to HPHCs:
    - COHb in blood,
    - Total NNAL in urine (expressed as concentration adjusted to creatinine).

- To describe the differences in self-reported perceptions and behaviors related to product use between THS users and former cigarette smokers compared to current cigarette smokers.

Endpoints:

- ABOUT–Perceived Risk (Health Risk) domain score
- ABOUT–Dependence total and domains scores (Extent of Use, Signs and Symptoms, Behavioral Impact), (between THS users and cigarette smokers only).

Study Hypotheses:

1. The primary study hypothesis is that there are beneficial differences in exposure to HPHCs (COHb and total NNAL), and also in inflammation (WBC), and in oxidative stress (8-epi-$PGF_{2\alpha}$), in THS users compared with current cigarette smokers.
2. The secondary study hypothesis is that there will be additional beneficial differences in lipid metabolism (HDL-C), or endothelial dysfunction (sICAM-1), or platelet activation (11-DTX-B_2), or additional functional benefits in arterial stiffness (AIx) or lung function (FEV_1 %predicted post-BD) in THS users compared with current cigarette smokers.

Evaluation Criterion:

The study will be declared successful if a beneficial difference is demonstrated on all endpoints tested to support the co-primary objectives (COHb, total NNAL, WBC and 8-epi-$PGF_{2\alpha}$), using a one-sided test-wise type I error level of 2.5%.

Additional benefits or functional benefits will be declared successful for each endpoint used for the evaluation of key secondary objectives (HDL-C, sICAM-1, 11-DTX-B_2, AIx, FEV_1 %predicted post-BD) for which a beneficial difference is demonstrated, using the Hochberg procedure for adjustment for multiplicity with a target of 2.5% one-sided test-wise type I error level.

Study Design:

This is a cross-sectional 3-group study with enrolled subjects matched by region (Asia, Europe), age (< 40, 40-50, > 50 years old), sex, and average daily product consumption over the last 2 years of smoking as self-reported (smoking 10 to 19 cig/day or using 10 to 19 heatsticks/day vs. > 19 cig/day or > 19 heatsticks/day).

The study will be conducted as a multi-center and multi-regional study, in Asia and Europe.

Enough subjects will be pre-screened in order to enroll 300 triplets. A triplet being defined as one current cigarette smoker, one THS user and one former cigarette smoker – enrolled and matched. Once 300 triplets have been completed, those subjects who have additionally been pre-screened will not be further invited for screening visits and will be informed that the recruitment for the study is complete. A maximum of 330 subjects per group will be enrolled – a 10% buffer is intended to ease triplet completion and manage mismatched subjects. A sufficient number of subjects will be screened in order to enroll them into 3 different groups as follows:

- A target of 300 current cigarette smokers,
- A target of 300 THS users with a minimum of 2 years of THS use,
- A target of 300 former cigarette smokers with minimum of 2 years of smoking abstinence.

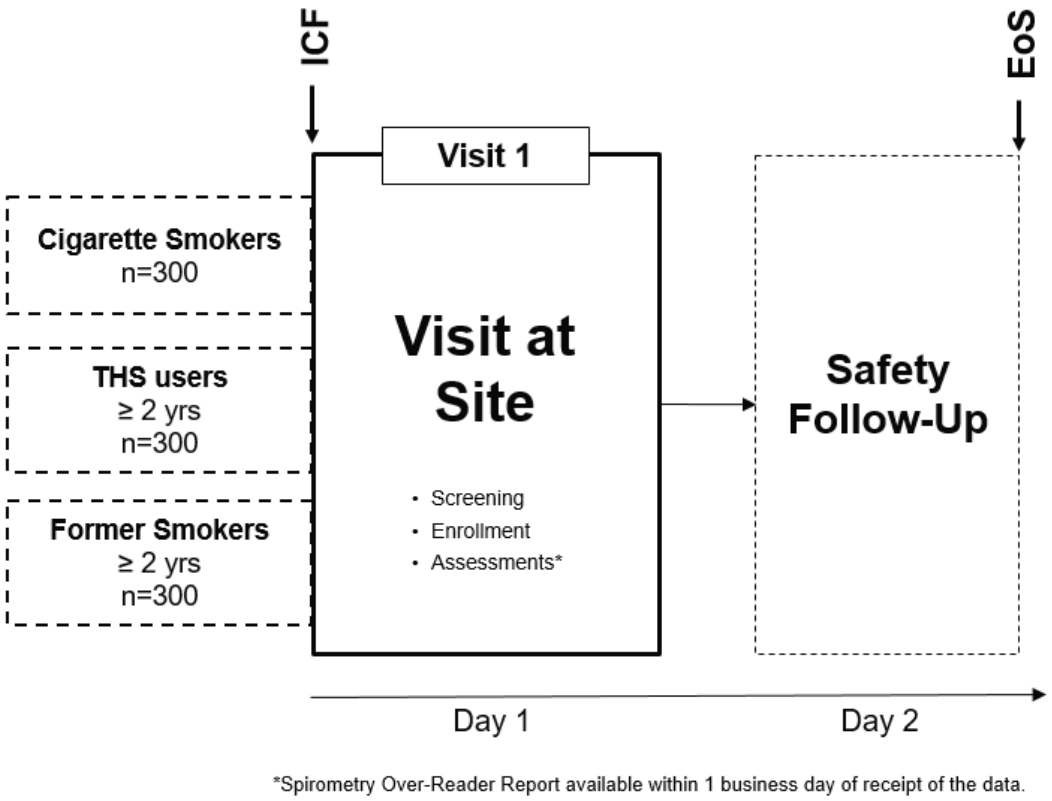

Figure 1 Study Flow Chart

The study will be conducted as follows:

- The Screening/Enrollment/Assessments Visit at Site: Visit 1 (Day 1)
- The Safety Follow-up (FU) period (Day 2)

Biobanking:

Blood, plasma and serum samples will be collected for long-term storage biobanking for future biomedical research such as identification of potential BoPHs (if the subject consents to optional ICF(s)).

Study Population and Main Criteria for Inclusion/Exclusion:

Asian and European female or male adult subjects with no restriction on race or ethnicity.

Inclusion criteria:

1. Subject is able to understand the information provided in the main ICF and has signed the main ICF.
2. Subject is 30-60 years old.
3. Subject is healthy based on ECG, spirometry, vital signs, physical examination, medical history and Investigator's assessment.

Cigarette smokers:

4. Has smoked ≥ 10 cigarettes/day on average (no brand restriction) over the past 2 years prior to screening.
5. Has smoked ≥ 10 cigarettes/day on average (no brand restriction) for at least 10 years.
6. Has not used other tobacco and nicotine products apart from cigarettes on a daily basis over the past 2 years prior to screening.
7. Smoking status will be verified by urinary cotinine test (≥ 200 ng/mL) and CO breath test (≥ 10 ppm (1)).

THS users:

8. Has used ≥ 10 HeatSticks/day on average over the past 2 years prior to screening.
9. Has smoked ≥ 10 cigarettes/day on average (no brand restriction) for at least 8 years prior to switching to THS.
10. Has smoked < 30 cigarettes/month and used other tobacco products or e-cigarettes < daily over the past 2 years prior to screening.
11. Product use will be verified by urinary cotinine test (≥ 200 ng/mL) and CO breath test (< 10 ppm).

Former cigarette smokers:

12. Has not smoked cigarettes or used any tobacco or nicotine-containing products on a daily basis over the past 2 years prior to screening.
13. Has smoked ≥ 10 cigarettes/day on average (no brand restriction) for at least 8 years prior to stopping smoking.
14. Smoking status will be verified by urinary cotinine test (< 100 ng/mL) and CO breath test (< 10 ppm).

Exclusion criteria:

1. As per the judgment of the Investigator, subject cannot participate in the study for any reason (*e.g.*, medical, psychiatric and/or social reason). The Investigator should specifically evaluate the subject's eligibility in light of COVID-19 risk factors and local situation.
2. Subject has/had clinically relevant diseases (including but not limited to gastrointestinal, renal, hepatic, neurological, hematological, endocrine, oncological, urological, immunological, pulmonary, and cardiovascular disease) or conditions that in the opinion of the investigator would jeopardize the safety of the subject or affect the validity of the study results.
3. Subject has/had within 30 days prior to screening a body temperature >37.5°C or an acute illness (e.g., upper-respiratory-tract infection, viral infection, etc.…) or the subject is confirmed or suspected of having active COVID-19 infection (based on the signs and symptoms observed at the time of assessment) at screening.
4. Subject has used any prescribed or over-the-counter systemic medication with an impact on WBC or 8-epi-$PGF_{2\alpha}$ within 5 half-lives of the medication prior to enrollment in the study (please refer to Appendix B).
5. Subject has high blood pressure (hypertension), defined as > 139 mmHg systolic and/or > 89 mmHg diastolic or is currently treated with medication controlling high blood pressure.
6. Subject who has (FEV_1/FVC) < 0.7 and FEV_1 < 80% predicted value at post-bronchodilator (BD) spirometry.
7. Subject who has (FEV_1/FVC) < 0.75 (pre-BD) and reversibility in FEV_1 (that is both > 12% and > 200 mL from pre- to post-BD values).
8. Subject who has a body mass index (BMI) < 18.5 or ≥ 30 kg/m^2.
9. Subject with positive alcohol and/or drug screening test results.
10. Pregnant or breast-feeding female subject.

Withdrawals and drop-outs will not be replaced.

Investigational Products; Dose; and Mode of Administration:

NA

Duration of Study:

The entire study duration per subject will be in general 2 days, including a 1-day visit at site followed by a 1-day FU Period. The study duration may be extended to maximum 4 days in case of lost to follow-up, including 2 days for additional calls. The EOS of the entire study is the last individual subject's EOS.

Subjects with ongoing AEs at the end of V1 should be contacted at the end of 1-day FU period. In case they cannot be reached, 2 additional attempts at the next 2 consecutive days will be made. If the subjects cannot be reached after the three attempts they will be declared lost to follow-up.

Statistical Methods:

Study endpoints will be listed and summarized for all subjects attending Visit 1 and in a subset of them excluding subjects with deviations impacting the evaluability of the primary objective.

The study hypothesis and related evaluation criterion are described above.

The overall, study-wise, type I error will be preserved at 2.5% 1-sided for both the primary and the key secondary objectives by:

- Testing simultaneously all the endpoints used for the evaluation of the primary objective as "co-primary endpoints" at a 2.5% 1-sided test-wise alpha level, and
- Testing the endpoints used for the evaluation of key secondary objectives only if the primary objectives has reached statistical significance, and using the Hochberg procedure for adjustment for multiplicity with a target of 2.5% 1-sided test-wise alpha level.

The analysis of all endpoints will be done using generalized linear mixed models adjusting notably but not limited to the matching variables, the interaction between the groups (THS users, former cigarette smokers, current cigarette smoker), the natural log time in the group (time in years since switching/quitting/smoking) and the study site as a random effect to compare THS users and former cigarette smokers to current cigarette smokers.

Modeling assumptions will be evaluated and model fit assessed by the analysis of residuals (model diagnostics) and by comparing the values predicted versus the observed endpoint values (calibration).

Sample Size:

Enrolling 300 cigarette smokers and 300 THS users, and assuming 95% of the subjects will be in the Modified Per-Protocol Analysis Set (285 in both groups), the study will provide more than:

- 99% power to demonstrate simultaneously a beneficial difference on all the endpoints used for the evaluation of primary objectives (COHb, total NNAL, WBC and 8-epi-$PGF_{2\alpha}$),
- 95% power to demonstrate a beneficial difference on the following key secondary objective endpoints: HDL-C, sICAM-1, 11-DTX-B_2,
- 73% power to demonstrate a functional benefit with the key secondary objective endpoint FEV_1 %predicted, post-BD,
- 53% power to demonstrate a functional benefit with the key secondary objective endpoint AIx

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ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviations

11-DTX-B_2	11-dehydrothromboxane B_2
ABOUT	Assessment of Behavioral OUtcome related to Tobacco and nicotine products
AE	Adverse event
AIx	Augmentation index
BD	Bronchodilator
BMI	Body mass index
BoExp	Biomarker of exposure
BoPH	Biomarker of potential harm
2CyEMA	2-Cyanoethyl Mercapturic Acid *N*-Acetyl-*S*-(2-cyanoethyl)-L-cysteine (2CyEMA)
CD	Compact disc
CI	Confidence interval
CO	Carbon monoxide
COHb	Carboxyhemoglobin
COPD	Chronic obstructive pulmonary disease
COVID-19	Coronavirus disease 2019
CRF	Case report form
CRO	Contract research organization
CSR	Clinical study report
CTMS	Clinical trial management system
CV (documentation)	Curriculum vitae
CV (statistics)	Coefficient of variation
CVD	Cardiovascular disease
DMP	Data management plan
DNA	Deoxyribonucleic acid
ECG	Electrocardiogram
8-epi-$PGF_{2\alpha}$	8-epi-Prostaglandin-$F_{2\alpha}$
ePRO	electronic patient reported outcome
EOS	End of study

FAS	Full analysis set
FDA	Food and Drug Administration
FEV_1	Forced expiratory volume in 1 second
FU	Follow-up
FVC	Forced vital capacity
GCP	Good Clinical Practice
HbA1c	Glycated Hemoglobin A1c
HCY	Homocysteine
HNBP	Heat not burn products
HPHCs	Harmful and potentially harmful constituents
hs-CRP	high-sensitivity C-Reactive Protein
ICF	Informed consent form
ICH	International Conference on Harmonization
IEC	Independent Ethics Committee
IL-6	Interleukin-6
IP	Investigational product
IRB	Institutional Review Board
LLOQ	Lower limit of quantification
MDA	Malondialdehyde
MedDRA	Medical dictionary for regulatory activities
MPO	Myeloperoxidase
mPP	Modified Per-Protocol Analysis Set
MRTP	Modified risk tobacco product
NEQ	Nicotine Equivalents
NNAL	4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol
PMI	Philip Morris International
PRO	Patient-reported outcome
PWV	Pulse Wave Velocity
QC	Quality control
SAE	Serious adverse event
SAP	Statistical analysis plan
SC	Smoking cessation
SHM	Sample handling manual

sICAM-1	Soluble intercellular adhesion molecule-1
SOP	Standard operating procedure
suPAR	soluble urokinase Plasminogen Activator Receptor
TC	Total cholesterol
TG	Triglycerides
THS	Tobacco Heating System
TNF-α	Tumor Necrosis Factor-α
TNP	Tobacco and/or nicotine containing product
UBC	United BioSource Corporation
ULOQ	Upper limit of quantification
VAS	Visual analogue scale
WBC	White blood cell (count)
WHO	World Health Organization
WHR	Waist-to-Hip ratio

Explanation of Terms

The following special terms are used in this protocol:

Cigarette	The term 'cigarette' refers to commercially available cigarettes (manufactured) and excludes THS with HeatSticks, cigars, cigarillos, e-cigarettes, hand-rolled cigarettes pipes, hookah, bidis, and other tobacco- or nicotine-containing products.
End of study	The EOS for a subject is defined as the end of the Safety Follow-up Period or the date of lost to follow-up. The EOS of the entire study is defined as the last individual subject's end of study.
Enrollment	At V1 for eligible subjects after all applicable entry criteria have been satisfactorily met.
Investigator	Principal Investigator, sub-Investigator or designee.
Lost to Follow-up	Subjects with ongoing AEs at the end of V1 should be contacted at the end of 1-day safety FU period. In case they cannot be reached, 2 additional attempts at the next 2 consecutive days will be made. If the subjects cannot be reached after the three attempts, they will be declared lost to follow-up.
Safety follow-up	After Discharge at V1, subjects will enter into a 1-day safety follow-up for the recording of spontaneously reported new AEs/SAEs and the follow-up of ongoing AEs/SAEs by the study site.
Screen failure	Subject who signs ICF but was not enrolled. Re-screening of subjects who did not meet entry criteria will not be permitted.

1 ETHICS AND REGULATIONS

1.1 Institutional Review Board (IRB)/Independent Ethics Committee (IEC) Approval

Prior to the start of the study, the clinical study protocol, together with its associated documents (informed consent forms [ICFs] including the subject information sheet, subject recruitment procedures [e.g., advertisements], written information to be provided to the subjects, curriculum vitae of the Investigators and/or other evidence of qualifications and any other documents requested by an IRB or IEC, will be submitted for review and approval to the relevant IRB/IEC in line with the applicable provisions of 21 Code of Federal Regulations (CFR) part 50 ("Informed Consent of Human Subjects") and 21 CFR part 56 ("Institutional Review Boards"). The IRB/IEC shall be appropriately constituted and perform its functions in accordance with the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) Guidance for Good Clinical Practice (GCP) (2) and local requirements, as applicable.

In accordance with GCP and 21 CFR part 56, a written confirmation of the IRB/IEC approval should be provided to the Sponsor. This should identify the study (name of the Investigators, study number, and title) and the documents that have been approved by the IRB/IEC, with dates and version numbers, as well as the date of approval. The composition of the IRB/IEC, including the name and occupation of the chairperson, will be supplied to the Sponsor together with a GCP compliance statement.

The written approval from the IRB/IEC will be filed in Investigator's file, and a copy will be filed in the study master file with the Sponsor or a designated organization. The study must not start at a site before the Sponsor has obtained written confirmation of favorable opinion/approval from the concerned IRB/IEC.

Any substantial change or addition to this protocol will require a written protocol amendment that must be approved by the Sponsor and the Investigators. All amendments will be submitted to the IRB/IEC, and substantial amendments will only be implemented after approval by the IRB/IEC.

These requirements for approval should in no way prevent any action from being taken by the Investigator or by the Sponsor in order to eliminate immediate hazards to the subjects. If such a change to the protocol is considered to be necessary by the Investigator, and is implemented for safety reasons, the Sponsor and the IRB/IEC should be informed immediately.

The Investigator is responsible for local reporting (e.g., to the IRB/IEC) of serious adverse events (SAEs) that occur during the study, according to local regulations.

Relevant safety information will be submitted to the IRB/IEC during the course of the study in accordance with national regulations and requirements.

Medically qualified study personnel will be available during the study. A separate optional ICF will be signed by the subject for the collection and storage of biobanking samples and their subsequent analysis.

1.2 Ethical Conduct of the Study

The study will be performed in accordance with ethical principles that have their origin in the Declaration of Helsinki (3) and will follow the principles as defined in the ICH GCP (2), in the Ministerial Ordinance on Good Clinical Practice for Drugs (Ministry of Health and Welfare) (4) and other applicable regulation.

The Investigator agrees to conduct the clinical study in compliance with the protocol agreed with the Sponsor and approved by the IRB/IEC. The Investigator and the Sponsor must sign the protocol (and protocol amendments, if applicable) to confirm this agreement. A copy of the Declaration of Helsinki is located in the Investigator's study file.

1.3 Subject Information and Consent

1.3.1 Informed Consent Form for Study Participation

Before or at V1, the Investigator will ensure that each subject is given full and adequate oral and written information about the nature, purpose, possible risks and benefits of the study, and the Investigator will answer all questions the subject might have to his/her full satisfaction. The subject will have sufficient time for consideration of his/her participation in the study and will be notified that he/she is free to withdraw his/her participation at any time.

Once the subject has received all the necessary information, and if he/she agrees to participate in the study, the subject and the person who conducted the informed consent discussion during V1 will both sign, date and time the ICF. The ICF includes both the subject information sheet and informed consent. No study-specific procedures will be performed before the ICF has been signed (including date and time).

The original, dated and signed ICF(s) must be kept by the Investigator and filed in the Investigator's file at the site or with the subject's files and a copy must be given to the subject.

The subject will be informed that if he/she withdraws from the study, the data collected until the point of withdrawal will be maintained as part of the study data and the samples collected prior to withdrawal will be analyzed unless subject's disagreement is documented at site. The subject will be informed that additional data analysis not mentioned in the protocol or in the statistical analysis plan (SAP) might be performed with the collected data at a later time. Any additional analyses performed will be covered by data confidentiality, as is the case with the main analysis described in this protocol.

1.3.2 Additional Informed Consent Forms for Optional Sample Collection

In addition to the ICF for study participation (main ICF), 2 separate additional ICFs will be distributed to each subject and asked to provide his/her optional consent for the collection of samples and storage for long-term biobanking at V1.

1. An ICF may be utilized to collect whole blood samples:
   - Biobank collection of whole blood for further transcriptomics profiling (mRNA & miRNA).
2. An ICF may be utilized to collect serum and plasma samples:
   - Biobank collection of serum and plasma samples for potential further measurements of BoExp, BoPH, proteomics, lipidomics, DNA methylation and metabolomics profiling. However, no DNA sequencing/genotyping will be performed.

Each subject will be given full and adequate oral and written information about the nature, purpose, possible risks and benefits of procedures included in the respective additional optional ICFs, and the Investigator(s) or designee(s) will answer all questions the subject might have to his/her full satisfaction. The subject will be notified that he/she is free to withdraw his/her consent for any additional ICF at any time. The additional subject's consent to collection of any samples for long-term storage in a biobank will be optional and will not be a requirement for his/her participation to the main study. The subject will be informed that additional statistical data analysis not mentioned in the protocol or in the statistical analysis plan (SAP) might be performed with the collected data at a later time. Any additional analysis performed will be also covered by data confidentiality, as it is for the main analysis described in this protocol.

Once the subject has received all the necessary information, and if he/she agrees to participate, this will be documented by the date, time and signature of both, the subject and the Investigator(s) or designee(s) who conducted the informed consent discussion.

1.3.3 Amendment to the Informed Consent Form

If a protocol amendment is required, an amendment to the ICF may be required. If a revision of the ICF is necessary, the Investigator will, with the support of the Sponsor, ensure that the documents have been reviewed and approved by a relevant IRB/IEC before ongoing subjects are required to re-sign the ICF (including date and time).

1.4 Good Clinical Practice and Regulatory Requirements

The procedures set out in this clinical study protocol pertaining to the conduct, evaluation, and documentation of this study are designed to ensure that the Sponsor, its authorized representative, Investigator abide by the principles of the ICH guidelines on GCP (2). These

guidelines apply specifically to pharmaceutical development, but nevertheless provide a robust and ethical framework for conducting clinical studies of tobacco products following the United States Food and Drug Administration (FDA) guidelines on modified risk tobacco product (MRTP) (5). The study will also be conducted in accordance with the general ethical principles outlined in the Declaration of Helsinki (3).

In addition, the Investigator will carry out the clinical study in accordance with applicable national and local laws of the pertinent regulatory authorities.

2 INTRODUCTION

2.1 Background

Smoking-related diseases have a complex etiology. Continuous exposure to harmful and potentially harmful constituents (HPHCs) affects multiple organ systems, disease pathways, and mechanisms occurring simultaneously, gradually leading to the development of smoking-related diseases over the course of years (6). There are multiple pathophysiological pathways underlying the development of smoking-related disease, i.e., lipid metabolism, inflammation, oxidative stress, endothelial function, platelet activation, transport of oxygen, lung function and carcinogenicity. The mortality rates attributable to smoking are for cardiovascular diseases (CVD), chronic obstructive pulmonary diseases (COPD), and lung cancer. An increase in inflammation and oxidative stress represents the initial alterations resulting from exposure to HPHCs during cigarette smoking, and is observed across the 3 main smoking-related-diseases (7).

Smoking is a known contributing factor to the development of atherosclerosis (8) and cardiovascular diseases (9, 10). The cardiovascular risk associated with smoking occurs in a non-linear, dose-dependent manner, with low levels of smoking (or passive smoking) still associated with a high excess risk (11, 12). The pathophysiological effects of smoking influences formation of atherosclerosis or narrowing of the arteries, leading to various types of CVD by affecting different vascular beds, such as ischemic heart disease, cerebrovascular disease, peripheral artery disease, and aortic aneurysm (11, 13, 14). To assess cardiovascular risk, the most common clinical outcomes are mortality and major cardiovascular morbidity (e.g., myocardial infarction, stroke and hospitalization due to angina). The rates of these clinical outcomes are, however, relatively low, particularly in populations with early-stage cardiovascular disease, which increases the sample size and duration in clinical trials significantly (15). To date there are several BoPH and surrogates that are representative of different pathways leading to atherosclerotic CVD described in the literature, that could be used to inform on further cardiovascular risk including endothelial dysfunction and injury, oxidative stress, hemostatic factors, fibrinolysis, inflammation, lipid modification, and vasomotor function (7, 13).

Endothelial dysfunction is the earliest detectable change in vascular health, and, importantly, it has consistently been shown to be associated with worsening cardiovascular risk (CV) and long-term outcomes (16, 17). Augmentation index (AIx), a functional assessment of endothelial dysfunction, also correlates to CV risk and may be a useful surrogate marker of arterial stiffness (18). In addition to AIx, arterial stiffness can be assessed by pulse wave velocity (PWV), another validated and independent predictor of CV risk (19). PWV measures the speed at which a pulse wave travels through the circulatory system, with higher values indicating stiffer arteries (20) .

In addition to the functional assessment of vascular dilation or stiffness, some BoPH have been shown to have good association with the mechanistic pathways leading to arterial dysfunction and CVD outcomes when smoking. In particular, as detailed in the 2004 Surgeon General's report, cigarette smoking causes systemic inflammation and oxidative stress (21). There is a large data set establishing that cigarette smoking significantly increases WBC counts, which is a sign of inflammation, and that there is a strong association between the level of WBC count and various CVD outcomes (22).

An increase in WBC count and 8-epi $PGF_{2\alpha}$ levels in sputum have been also suggested to be associated with severity of COPD (23-25). In addition, a study carried out on more than 420'000 subjects suggested a clear association between increased count of WBC and incidence of lung cancer risk in UK (26). Similarly, urinary 8-epi $PGF_{2\alpha}$ levels were also suggested to be associated with an increased risk for lung cancer (27) and breast cancer (28).

The best way for smokers to reduce the CV risks of smoking is to quit. For those smokers who would otherwise continue smoking, Philip Morris International (PMI) is offering alternative approaches by developing products with the potential to reduce the risks of tobacco-related diseases. These products aim to substantially reduce or eliminate the exposure to harmful and potentially harmful constituents (HPHCs) found in cigarette smoke, apart from nicotine, while providing an acceptable substitute for cigarettes. One of these products is Tobacco Heating System (THS), developed by PMI, marketed under the name of IQOS and used with tobacco HeatSticks. It consists of an alternative tobacco product in which tobacco is heated to a lower temperature (350°C) than cigarettes (<800°C), thereby preventing the combustion process from occurring and producing significantly lower levels of known HPHCs compared with cigarette smoke.

PMI has undertaken a comprehensive assessment program on THS, including pre-clinical and clinical studies, aiming to demonstrate that THS is a reduced risk product (RRP)[3]. The non-clinical assessment of THS, consisting of the aerosol chemistry analysis, *in vitro* and *in vivo* studies, supported the initiation of clinical studies, as no new or increased toxicological hazard in the product's aerosol was detected when compared with cigarette smoke. Results from pre-clinical *in vivo* studies comparing cigarette smoke with THS aerosol in continuous inhalation showed that exposure to THS aerosol, at multiple concentrations, resulted in a dramatically lower systemic toxicity compared to cigarette smoking (29, 30). Furthermore, exposure to THS aerosol in mice did not enhance CV disease, unlike cigarette smoke, and switching from cigarette smoke to THS aerosol exposure halts, for example, aortic plaque growth in a similar manner as observed with smoking cessation (31).

[3] Reduced risk products ("RRPs") is the term used by PMI to refer to products that present, are likely to present, or have the potential to present less risk of harm to smokers who switch to these products versus continued smoking

Several clinical studies have been conducted with THS, in Europe, Asia and the United States, in order to evaluate the nicotine pharmacokinetics (PK) profile (32-35) to demonstrate reduced exposure (36-39), and to determine functional and biological changes when adult smokers switch from cigarettes to THS use compared to smokers continuing smoking cigarettes (38, 39) . The PK studies demonstrated similar nicotine absorption in both subjects using THS and subjects smoking cigarettes. The Reduced Exposure studies showed reductions in the levels of biomarkers of exposure (BoExp) to selected HPHCs, in subjects using THS compared to subjects continuing smoking cigarettes that approached levels observed when subjects stopped smoking for the duration of the study, both in controlled and ambulatory settings, for a duration of up to 3 months. These studies also indicated favorable biological and functional changes in clinical risk endpoints linked to smoking-related diseases.

A 6-month exposure study (40), followed by a 6-month extension study (41), conducted in U.S., showed beneficial changes in BoPH similar when contextualized to what is observed upon smoking cessation in smokers switching from cigarettes to THS compared to smokers continuing smoking cigarettes. In particular, this study showed a decrease in WBC count and 8-epi $PGF_{2\alpha}$ in predominant THS users (using more than 70% of THS sticks) as compared to smokers who continued smoking cigarettes (42). Post-marketing studies are ongoing, in order to have a better understanding of the product use behaviors and first insights in health outcomes (43). Safety data available to date have shown a similar short-term safety profile for THS than for cigarettes. Further product information can be found in the SPI (44).

Because cigarette smoking leads to a pro-inflammation state and oxidative stress and that smoking cessation can reverse these alterations, a beneficial impact on these 2 components in users of THS for at least 2 years would provide additional substantiation in favor of THS as an alternative product to cigarette that could reduce the harm of healthy smokers for multiple diseases long-term. Considering our previous positive results in the American population, the study proposed here intends to verify the beneficial impact of THS use versus continuing cigarette smoking on BoPH in European and Asian population when contextualizing results to former smokers. In addition, the study intends to show that beneficial impact on BoPH with long term use of THS translates also in benefits for smokers on cardiovascular and respiratory functions.

2.2 Purpose of the Study

The purpose of this study is primarily to demonstrate beneficial effects of switching from cigarettes to THS for at least 2 years compared to cigarette smoking in a real-life condition on both inflammation and oxidative stress status as a proxy for further long-term harm in healthy subjects, using the well-established and fit-to-purpose measures of WBC and 8-epi-$PGF_{2\alpha}$, respectively, as indicators of the status of these pathways.

The study aims also at demonstrating additional benefits on other mechanistic pathways along with inflammation and oxidative stress by the means of additional BoPH and to assess association with functional benefits that are expected to be responsive to the extent of exposure to HPHCs. This will include:

- BoPH associated with lipid metabolism, endothelial dysfunction, and platelet activation,
- Functional endpoints associated with arterial stiffness, and lung function,
- BoExp to HPHCs

2.3 Anticipated Benefits and Risks

2.3.1 Anticipated Benefits

Advice on health risks associated with smoking, and smoking cessation advice will be provided at Visit 1. The advice will follow the recommendations of the World Health Organization (WHO) "Evidence based Recommendations on the Treatment of Tobacco Dependence" (45) or the recommendations of the Japanese Circulation Society (46), and of the Japanese Ministry of Health, Labor and Welfare (47), depending on the country.

2.3.2 Anticipated Foreseeable Risks due to Study Procedures

The risks related to study procedures (e.g., blood samples) are deemed to be part of procedures routinely performed during normal or extended health examinations by the subject's health care professional.

PWV assessment utilizes applanation tonometry where pressure is partially applied to the carotid artery, compressing the vessel against the bone, tissue & ligaments beneath that may induce a baroreceptor response. However, the theoretical risk of stimulating carotid baroreceptors through neck compression is very small (48).

Administration of salbutamol/albuterol for the spirometry testing may potentially elevate blood pressure, increase the heart rate and cause tremor, inner agitation, palpitation due to sinus tachycardia, muscle cramps or headaches. However, these effects are limited after single use and only more frequent following repeated use and oral administration. Salbutamol/albuterol should not be administered to subjects whose blood pressure and/or heart rate is already markedly elevated and whose heart rhythm is already irregular.

3 STUDY OBJECTIVES

3.1 Primary Objective and Endpoints

The primary objective of this study is:

1. To demonstrate beneficial differences in inflammation, oxidative stress, and exposure to Harmful and Potentially Harmful Constituents (HPHCs) in THS users compared with current cigarette smokers.

   Endpoints:

   - Biomarkers of Exposure (BoExp) to HPHCs:
     - Carboxyhemoglobin (COHb) in blood.
     - Total 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (total NNAL) in urine (expressed as concentration adjusted to creatinine),
   - Biomarkers of Potential Harm (BoPH) for core pathophysiological pathways underlying the main smoking-related diseases:
     - Inflammation: White Blood Cell total count (WBC) in blood,
     - Oxidative stress: 8-epi-Prostaglandin-$F_{2\alpha}$ (8-epi-$PGF_{2\alpha}$) in urine (expressed as concentration adjusted to creatinine).

3.2 Secondary Objectives and Endpoints

3.2.1 Key Secondary Objectives and Endpoints

The key secondary objectives of this study are:

1. To demonstrate beneficial differences in lipid metabolism, endothelial dysfunction, or platelet activation between THS users and current cigarette smokers.

   Endpoints:

   - Lipid metabolism: High-Density Lipoprotein Cholesterol (HDL-C),
   - Endothelial dysfunction: soluble Intercellular Adhesion Molecule-1 (sICAM-1)
   - Platelet activation: 11-dehydrothromboxane B2 (11-DTX-B_2),

2. To demonstrate functional benefits in THS users compared to current cigarette smokers.

   Endpoints:

   - Arterial stiffness: Augmentation Index (AIx),

- Lung function: Forced Expiratory Volume in 1 second (FEV_1) %predicted, post-bronchodilator (post-BD)

3.2.2 Other Secondary Objectives and Endpoints

The other secondary objectives of this study are:

1. To determine the differences in BoPH between THS users and former cigarette smokers compared to current cigarette smokers.

   Endpoints:

   - Inflammation:
     - Neutrophil to Lymphocyte Ratio (NLR),
     - High-sensitivity C-Reactive Protein (hs-CRP),
     - Homocysteine (HCY).
   - Oxidative stress:
     - Myeloperoxidase (MPO).
   - Lipid metabolism:
     - Triglycerides (TG).
   - Platelet Activation:
     - Fibrinogen.
   - Glycemic Control:
     - Glycated Hemoglobin (HbA1c).
   - Lung function (spirometry with and without bronchodilator, absolute and % predicted values, where applicable):
     - FEV_1/FVC.

2. To determine the differences in the levels of BoExp to nicotine between THS users and former cigarette smokers compared to current cigarette smokers.

   Endpoints:

   - BoExp to nicotine:
     - Nicotine Equivalents (NEQ) in urine (expressed as concentration adjusted to creatinine),
     - 2-Cyanoethyl Mercapturic Acid *N*-Acetyl-*S*-(2-cyanoethyl)-L-cysteine (2CyEMA) in urine (expressed as concentrations adjusted to creatinine).

3. To determine the differences in self-reported health perceptions between THS users and former cigarette smokers compared to current cigarette smokers.

   Endpoints:

   - Short Form Health Survey (SF-36) domain scores (Physical Functioning and Mental Health),
   - ABOUT–Health and Functioning domain scores (between THS users and cigarette smokers only).

4. To evaluate the safety during the study.

   Endpoints:

   - Adverse events (AEs),
   - Serious adverse events (SAEs).

3.3 Exploratory Objectives and Endpoints

The exploratory objectives of this study are:

1. To describe the differences in additional BoPH between THS users and former cigarette smokers compared to current cigarette smokers.

   Endpoints:

   - Inflammation:
     - WBC (between cigarette smokers and former cigarette smokers only)
     - Interleukin-6 (IL-6),
     - Tumor Necrosis Factor-α (TNF-α),
     - soluble urokinase Plasminogen Activator Receptor (suPAR).
   - Oxidative stress:
     - 8-epi-PGF2α (between cigarette smokers and former cigarette smokers only),
     - Malondialdehyde (MDA).
   - Lipid metabolism:
     - HDL-C (between cigarette smokers and former cigarette smokers only),
     - Total Cholesterol (TC),
     - Low-Density Lipoprotein Cholesterol (LDL-C),
     - oxidized Low-Density Lipoprotein (ox-LDL),

- Endothelial Dysfunction:
  - sICAM-1 (between cigarette smokers and former cigarette smokers only),
- Platelet Activation:
  - 11-DTX-B_2 (between cigarette smokers and former cigarette smokers only),
- Arterial Stiffness:
  - AIx (between cigarette smokers and former cigarette smokers only),
  - Pulse Wave Velocity (PWV).
- Lung Function (spirometry with and without bronchodilator, absolute and % predicted values, where applicable):
  - Forced Vital Capacity (FVC),
  - FEV_1, (FEV_1 %predicted post-BD between cigarette smokers and former cigarette smokers only)
  - Forced Expiratory Flow between 25% and 75% of FVC ($FEF_{25-75\%}$).

2. To describe the differences in the levels of BoExp to HPHCs and nicotine between cigarette smokers and former cigarette smokers.

   Endpoints:

   - BoExp to HPHCs:
     - COHb in blood,
     - Total NNAL in urine (expressed as concentration adjusted to creatinine).

3. To describe the differences in self-reported perceptions and behaviors related to product use between THS users and former cigarette smokers compared to current cigarette smokers.

   Endpoints:

   - ABOUT–Perceived Risk (Health Risk) domain score
   - ABOUT–Dependence total and domains scores (Extent of Use, Signs and Symptoms, Behavioral Impact), (between THS users and cigarette smokers only).

4 INVESTIGATIONAL PLAN

4.1 Overall Study Design and Plan

This is a cross-sectional 3-group study with subjects enrolled matched by region (Asia, Europe), age (< 40, 40-50, > 50 years old), sex, and average daily product consumption over the last 2 years as self-reported (smoking 10 to 19 cig/day or using 10 to 19 heatsticks/day vs. > 19 cig/day or > 19 heatsticks/day).

The study will be conducted as a multi-center and multi-regional study, in Asia and Europe as regions.

Both regions should have a quota applied to ensure representatives of each constitute at least 40% of the subjects in each group.

Both sexes should have a quota applied to ensure representatives of each constitute at least 40% of the subjects in each group.

Enough subjects will be pre-screened in order to enroll 300 triplets. A triplet being defined as one current cigarette smoker, one THS user and one former cigarette smoker – enrolled and matched. Once 300 triplets have been completed, those subjects who have additionally been pre-screened will not be further invited for screening visits and will be informed that the recruitment for the study is complete. A maximum of 330 subjects per group will be enrolled – a 10% buffer is intended to ease triplet completion and manage mismatched subjects. A sufficient number of subjects will be screened to enroll them into 3 different groups as follows:

- A target of 300 current cigarette smokers,
- A target of 300 THS users with a minimum of 2 years of THS use,
- A target of 300 former cigarette smokers with minimum of 2 years of smoking abstinence.

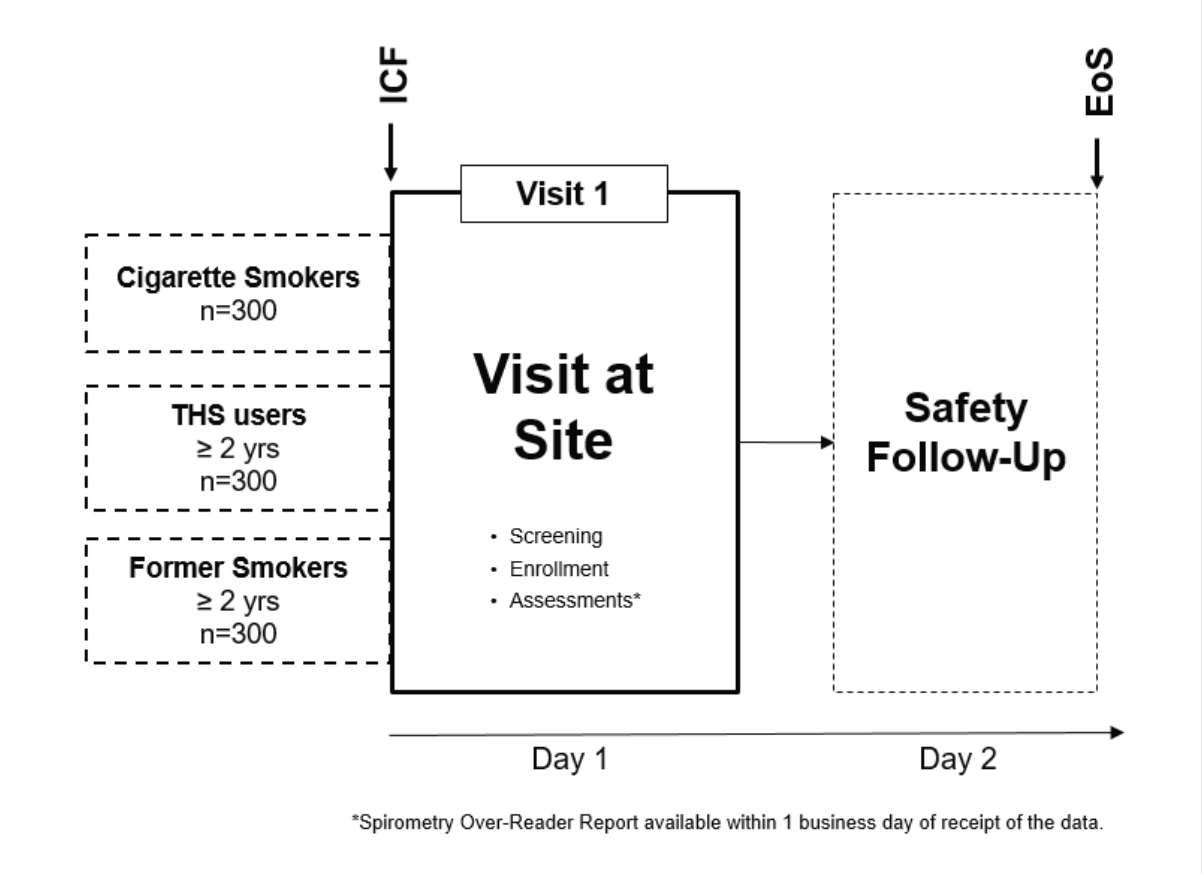

Figure 2 Study Flow Chart

The study will be conducted as follows:

- The Screening/Enrollment/Assessments Visit at Site: Visit 1 (Day 1),
- The Safety Follow-up (FU) period (Day 2).

Online Pre-screening and matching

Potential study subjects will be reached through social media and traditional display advertising, such as THS customer database/emailing campaign, and sites' database, as/if approved by IEC/IRB. After registration on the study web platform, they will be asked some questions in an online pre-screening phase about their age, sex, and tobacco/nicotine-containing products use history. Their answers from the online pre-screening will not be recorded in the Case Report Form (CRF). In case potential subjects meet the pre-screening criteria and have consented to share their personal information with an investigational site, they will be invited on site and asked more specific questions about their tobacco/nicotine-

containing products use history before enrollment. Their answers will be recorded in the CRF. Current cigarette smokers and former cigarette smokers will be matched with THS users by region, age, sex, and average daily THS used or cigarettes smoked over the last 2 years or cigarettes smoked before cessation as self-reported during the pre-screening. For each potential triplet of matched subjects, the THS user will be called first to attend Visit 1, while the current cigarette smoker and former cigarette smoker subjects of the triplet will be retained until THS user has performed Visit 1. Thereafter, the current cigarette smoker and former cigarette smoker will be called to attend Visit 1. If the triplet is incomplete at the time THS user performed Visit 1, all efforts will be done to complete the triplet.

Screening/Enrollment/Assessments Visit 1

The investigational sites will implement a risk minimization and mitigation plan for COVID-19, including precautions such as use of personal protective equipment for subjects, site staff and other visitors, site staff health-check, and disinfection of site premises. In addition, subject's body temperature measurements should be performed first and precede any other study assessment.

Subjects will sign the main ICF before the start of any procedure. During Visit 1, eligibility of the subjects to participate in the study will be assessed.

Subjects who meet all eligibility criteria will be enrolled. Subjects who do not meet the eligibility criteria will be considered as screen failures.

All other procedures and data collection will be completed following the enrollment of the sub as per schedule of events (Appendix A).

The FU Period (from Discharge at Visit 1 until the End of the FU Period)

After Discharge at Visit 1, the subject will enter a 1-day FU Period during which AEs reported by the subjects will be collected and follow-up of the ongoing AEs at discharge will be conducted by investigational site (section 8.2.5).

The individual end of study (EOS) date for a subject is defined as the end of FU Period or the date of lost to follow-up.

The EOS of the entire study is defined as the last individual subject's EOS.

4.2 Rationale for Study Design

This study aims to demonstrate favorable differences in 2 BoPH as indicators of inflammation (WBC) and oxidative stress (8-epi-$PGF_{2\alpha}$) as well as 2 BoExp to HPHCs (COHb and total NNAL) following at least 2 years of switching to THS use versus current cigarette smokers. These are pathways that are commonly altered by cigarette smoking across the 3 main smoking-related diseases (CVD, COPD and lung cancer). Smoking cessation has proven

beneficial on these pathways as well as on the risk of these diseases in smokers. Additional benefits will be demonstrated on key BoPH representative of pathomechanisms and functional endpoints (arterial stiffness and lung function) associated with CVD and COPD between THS users and current cigarette smokers to provide additional scientific evidence and strengthen the primary objective. General perceptions of health will be also assessed. To contextualize the results from THS users, endpoints will be also assessed in former cigarette smokers.

In 2007, a cross-sectional study in Japan, showed significant favorable differences in several cardiovascular BoPH, including WBC and 8-epi-$PGF_{2\alpha}$, between cigarette smokers and non-smokers (49). In 2008, a 12-month randomized controlled study showed significant favorable changes in several cardiovascular BoPH, including WBC in cigarette smokers switching to the second-generation electrically heated cigarette smoking system (THS prototype) (50). Recently, a 6-month exposure-response study conducted in USA showed that reduction in exposure to toxicants led to statistically significant changes in 5 of 8 BoPH (including WBC and 8-epi-$PGF_{2\alpha}$), when switching completely to THS compared to smoking cigarettes (42). These beneficial changes were maintained at 12 months of THS use (41). Increases in both WBC and 8-epi-$PGF_{2\alpha}$ from smoking was shown to be reversible, as shown in a large number of studies reporting a statistically significant decline in WBC count following smoking cessation (51). A very accurate measure of oxidative stress that is associated with diseases such as myocardial infarction, hypercholesterolemia and ischemic heart disease is 8-epi-$PGF_{2\alpha}$ (52). Levels of 8-epi $PGF_{2\alpha}$ have been reported to rapidly decline upon smoking cessation (53-56).

This study will provide additional evidence on the potential of THS to reduce the risk in smokers, collecting also data from countries (European and Asian) beyond the U.S. Furthermore, this study will inform on how BoPH modifications translate into cardiovascular and respiratory functional benefits and general well-being perceived by the in THS users versus current smokers. A cross sectional design was selected as it offers the advantage to further demonstrate the “reduced risk” potential of THS use versus cigarette smoking in real-life conditions “as the product is actually used”, when benchmarked to former smokers. For representativeness of the results, the country selection took into consideration both IQOS marketing date to ensure a sufficient number of subjects using THS for at least 2 years and meaningful product uptake.

- the rationale for choosing WBC and 8-epi-$PGF_{2\alpha}$ as endpoints was based on multiple criteria: the evidence that cigarette smoking increases inflammatory markers and oxidative stress (6, 21, 57).
- they are associated with COPD severity and lung cancer (23-27).
- smoking cessation has beneficial impact on their levels (58, 59).

The acute cardiovascular effect of cigarette smoking on the transport of hemoglobin via COHb has also been demonstrated in smokers who predominantly switch to THS for 6 months (42).

High levels of total NNAL, a BoExp to NNAL has been reported to be linked with the incidence of lung cancer (60).

The choice of the secondary endpoints is based as a selection of widely published BoPH representing different mechanistic pathways, all contributing to different pathophysiological mechanisms that are linked to the main smoking-related diseases (61-64).

From a cardiovascular perspective, AIx was selected as a key secondary endpoint since it reflects arterial stiffness (65, 66), is a cardiovascular risk marker and an independent predictor of cardiovascular events (20). From a respiratory perspective, FEV_1 %predicted is a diagnostic BoPH for COPD and slower decline in FEV_1 % predicted has been demonstrated in smokers who switch from cigarette to THS for 6 months predominantly (42). Several studies have assessed the effects of smoking cessation on AIx. Longitudinal smoking cessation studies has shown marked improvements in AIx with reduced values, while observational cross-sectional studies have reported less evidence for an association between smoking cessation and AIx. In former cigarette smokers, the duration of smoking cessation seems to correlate with the improvement of arterial stiffness parameters (65).

BoExp to nicotine (NEQ) and acrylonitrile (2CyEMA) will serve as indicators of exposure of the subjects prior to the visit at site. Levels of NEQ are expected to be lower in former cigarette smokers than in current cigarette smokers or THS users. Because nicotine is delivered by THS at levels comparable to cigarettes, NEQ levels are not expected to differ between THS users and cigarette smokers, but they will serve as an overall estimate of exposure to nicotine. Spot-urine collection will be performed, and the BoExp levels will be adjusted to creatinine to normalize urinary excretion rates.

Self-reported tobacco/nicotine use patterns, perceived risk, perceived dependence and general perceptions of health associated with tobacco/nicotine product use will be assessed via questionnaires to provide evidence on perception and behaviors of users of THS, current cigarette smokers, and former cigarette smokers.

The minimum age of 30 years old in the inclusion criteria was selected based on the legal age of smoking in some of the chosen countries (20 years old) and to account for the 10 years of smoking history.

The maximum age of 60 years old in the inclusion criteria was selected because it has been reported that associations between AIx and cardiovascular risk factors differed by age in subjects above 60 years old (67).

4.3 Appropriateness of Measurements

The BoPH selected in this study will be measured with valid and robust analytical methods that are practical and acceptable by subjects.

All ABOUT Toolbox self-report measures to be used in this study have been developed following the best practices (including the FDA's Guidance for Industry Patient-Reported Outcome (PRO) Measures (68)), which provide the scientific basis for the development, modification, and validation of PRO measures in support of clinical and regulatory research. Other generic self-reported measures that are included for concurrent validity purpose with the ABOUT Toolbox have been similarly validated.

The qualitative food group frequency, lifestyle, and socioeconomic questionnaires to be used in the study, are established and fit-for-purpose for the assessment of individual characteristics.

All self-report measures and questionnaires will be translated following appropriate linguistic validation guidelines.

4.4 Study Duration

The entire study duration per subject will be in general 2 days, including a 1-day visit at site followed by a 1-day FU Period. The study duration may be extended to maximum 4 days in case of lost to follow-up, including 2 days for additional calls. The EOS of the entire study is the last individual subject's EOS.

5 STUDY POPULATION

Asian and European female or male adult subjects with no restriction on race or ethnicity.

5.1 Selection of Study Population

5.1.1 Inclusion Criteria

Subjects who meet all the following inclusion criteria can be enrolled into the study:

1. Subject is able to understand the information provided in the main ICF and has signed the main ICF.
2. Subject is 30-60 years old.
3. Subject is healthy based on ECG, spirometry, vital signs, physical examination, medical history and Investigator's assessment.
4. Subject is ready to comply with the study procedures.

Cigarette smokers:

5. Has smoked ≥ 10 cigarettes/day on average (no brand restriction) over the past 2 years prior to screening.
6. Has smoked ≥ 10 cigarettes/day on average (no brand restriction) for at least 10 years.
7. Has not used other tobacco and nicotine products apart from cigarettes on a daily basis over the past 2 years prior to screening.
8. Smoking status will be verified by urinary cotinine test (≥ 200 ng/mL) and CO breath test (≥ 10 ppm (1)).

THS users:

9. Has used ≥ 10 HeatSticks/day on average over the past 2 years prior to screening.
10. Has smoked ≥ 10 cigarettes/day on average (no brand restriction) for at least 8 years prior to switching to THS.
11. Has smoked < 30 cigarettes/month and used other tobacco products or e-cigarettes < daily over the past 2 years prior to screening.
12. Product use will be verified by urinary cotinine test (≥ 200 ng/mL) and CO breath test (< 10 ppm).

Former cigarette smokers:

13. Has not smoked cigarettes or used any tobacco or nicotine products on a daily basis over the past 2 years prior to screening.
14. Has smoked ≥ 10 cigarettes/day on average (no brand restriction) for at least 8 years prior to stopping smoking.
15. Smoking status will be verified by urinary cotinine test (< 100 ng/mL) and CO breath test (< 10 ppm).

5.1.2 Exclusion Criteria

Subjects who meet any of the following exclusion criteria must not be enrolled into the study:

1. As per the judgment of the Investigator, the subject cannot participate in the study for any reason (*e.g.*, medical, psychiatric and/or social reason). The Investigator should specifically evaluate the subject's eligibility considering COVID-19 risk factors and local situation.
2. The subject is legally incompetent or physically/mentally incapable of giving consent (e.g., emergency situation, under guardianship, in a social or sanitary establishment, prisoner or involuntarily incarcerated).
3. The subject has/had clinically relevant diseases (including but not limited to gastrointestinal, renal, hepatic, neurological, hematological, endocrine, oncological, urological, immunological, pulmonary, and cardiovascular disease) or conditions that in the opinion of the investigator would jeopardize the safety of the subject or affect the validity of the study results.
4. The subject has abnormal findings on physical examination, ECG, vital signs, spirometry or in the medical history, deemed clinically significant by investigators.
5. The subject has/had within 30 days prior to screening a body temperature >37.5°C or an acute illness (e.g., upper-respiratory-tract infection, viral infection, etc.…) or the subject is confirmed or suspected active COVID-19 infection (based on the signs and symptoms observed at the time of assessment) at screening.
6. The subject has used any prescribed or over-the-counter systemic medication with an impact on WBC or 8-epi-$PGF_{2\alpha}$ within 5 half-lives of the medication prior to enrollment in the study (please refer to Appendix B).
7. Subject has high blood pressure (hypertension), defined as > 139 mmHg systolic and/or > 89 mmHg diastolic or is currently treated with medication controlling high blood pressure.
8. The subject has (FEV_1/FVC) < 0.7 and FEV_1 < 80% predicted value at post-bronchodilator (BD) spirometry.

9. The subject has (FEV_1/FVC) < 0.75 (pre-BD) and reversibility in FEV_1 (that is both > 12% and > 200 mL from pre- to post-BD values).
10. The subject has a history of allergic reactions to salbutamol.
11. The subject has a body mass index (BMI) < 18.5 or ≥ 30 kg/m^2.
12. The subject has positive alcohol and/or drug screening test results.
13. The subject has donated or received whole blood or blood products within 3 months prior to V1.
14. The subject has been previously screened for this study.
15. The subject is a current or former employee of the tobacco or e-cigarettes industry or of their first-degree relatives (parent, sibling, and child).
16. The subject is an employee of the investigational site or any other parties involved in the study or of their first-degree relatives (parent, sibling, and child).
17. The subject has participated in a clinical study within 3 months prior to V1.
18. For women only: the subject is pregnant (does have a positive pregnancy test) or breast-feeding.

Withdrawals and drop-outs will not be replaced.

5.2 Discontinuation of Subjects from the Study

Discontinued subjects will include both, subjects who withdraw from the study (subject's decision) and subjects who are discontinued from the study by the decision of the Investigator. A subject can only be discontinued from the study after enrollment.

Subjects will be informed that they are free to withdraw from the study at any time. Subjects should be questioned for the reason of premature withdrawal from the study, although they are not obliged to disclose it.

If the subject withdraws his/her consent for the study (main ICF), the Investigator needs to document whether, if applicable, the subject still consents for long-term biobanking. This information needs to be fully documented in the source document and CRF.

Subjects discontinued from the study cannot re-enter the study.

Discontinuation from the study

Subjects must be discontinued from the study for any of the following reasons:

1. Withdrawal of informed consent.

2. Discontinuation is considered to be in the interest of the subject from a safety perspective as judged by the Investigator.
3. The Sponsor or the Investigator terminates the study. If the Sponsor or the Investigator decides to prematurely terminate the study, the subject will be promptly informed. The Investigator should report the fact and the reason of termination in writing to the IRB or IEC.

Subjects may be discontinued from the study for the following reason on the judgment of the Investigator:

1. Non-compliance to the study procedures.

5.3 Lost to Follow-up

Subjects with ongoing AEs at the end of V1 should be contacted at the end of 1-day FU period. In case they cannot be reached, 2 additional attempts on the next 2 consecutive days will be made. If the subjects cannot be reached after the three attempts they will be declared lost to follow-up. All the attempts should be recorded in the source documents.

5.4 Violation of Selection Criteria

Any subject who does not meet the entry criteria after signing the ICF and prior to enrollment, will be considered as screen failure. Re-screening of subjects will not be permitted.

Given the study duration (two days), it is unlikely that violation of selection criteria will be detected during study. In such cases subjects will not be discontinued.

6 INVESTIGATIONAL PRODUCTS

6.1 Description of Investigational Products

Not applicable.

6.2 Use of Investigational Product(s)

Not applicable.

6.3 Blinding

This is an open-label study; therefore, the subjects and Investigators will be unblinded to the subject's group. However, there will be a limited degree of blinding in the data review and data analysis process. In particular, PMI and contract research organization (CRO) personnel will be blinded to the endpoints tested in the primary objective, to the groups and other data whose value or solely availability may be indicative of the group allocation, as summarized in Table

Table 1 Description of Blinded Study Personnel

Blinded Study Personnel	Blinded Data[a]	End of Blinding Period
• PMI and CRO Study Statisticians • PMI Clinical Scientist	Group for: • All endpoints All data for: • Endpoints as part of the primary and key secondary objectives • Questions on tobacco/nicotine-containing products use history • CO breath test • Cotinine tests • BoExp to HPHCs/nicotine • Inclusion/exclusion criteria • ABOUT–Perceived risk • ABOUT–Dependence • ABOUT-Health and Functioning	After the SAP finalization.

[a] Blinded data will be made accessible to the blinded personnel by means of a dummy group or masking. As part of the PMI quality control (QC) activity, data listings will be reviewed by the CRO and PMI before database lock, with no access to the group information. Full details will be available in the data review plan.

Any PMI and CRO personnel who are not listed in the above table will be unblinded by default.

Unblinded information will not be shared with the blinded study team, until the end of the blinding period (Table 1). PMI will receive blinded and unblinded data for the pre-analysis data review as planned in the data review plan. Blinded data will be accessible by the blinded study personnel in a masked format or presented independent of the subject identifier so to ensure that data cannot be associated to a subject. Unblinded data will only be reviewed by the unblinded study team.

6.4 Restrictions

6.4.1 Product Use Restrictions

No smoking or THS use on site will be allowed. Subjects will not be allowed to smoke cigarettes or use THS for 2 hours before the AIx and PWV measurements, 1h before spirometry assessment and 15 min before vital signs assessment.

6.4.2 Dietary Restrictions

Subjects will not be allowed to bring their own food or beverages to the site. During Visit 1, light snacks, fruits and raw vegetables might be distributed to the subjects without restrictions at any time during the visit. Consumption of water will be allowed as desired. Subjects should also refrain from consuming large meals, and caffeine-containing food (e.g., chocolate)/drinks for at least 3 hours before the AIx and PWV measurements. Spirometry will also be conducted at least 2 hours after eating.

7 STUDY PROCEDURES

Personnel performing study assessments must have the appropriate and fully documented training. An overview of all study assessments is shown in the schedule of events (Appendix A). In this section, only the expected/planned timepoints for the various assessments are described. Appropriate medical advice will be provided to the subject in case of any medical findings requiring health care. Site personnel will adhere to the site's standard operating procedures (SOPs) for study related procedures.

7.1 Informed Consent

Prior to any study assessment being performed, the subject will be asked to provide his/her written consent to participate in the study (ICF for study participation/main ICF) (section 1.3.1). Apart from pre-screening activities described in Section 4.1, all the assessments must start only after the time of the main ICF signature by the subject.

The consent(s) will be captured in the CRF.

7.2 Information on the Risk of Smoking and Smoking Cessation Advice and Debriefing

At V1, each subject will be given information on the risks of smoking and smoking-cessation advice. The information on the risk of smoking and the advice on smoking cessation will take the form of a brief interview according to the recommendations of the World Health Organization (WHO) guidelines (45).

7.3 Clinical Assessments

7.3.1 Demographic Data

At V1, sex, age and race will be recorded.

7.3.2 Medical History, Concomitant Disease, Previous and Concomitant Medications

Relevant medical history and any concomitant disease will be documented at V1. Medical history is defined as any condition that started and ended prior to V1. A concomitant disease is defined as any condition that is either detected at V1, and/or is still ongoing at V1.

All medication taken 4 weeks prior to V1 and all concomitant medication taken during the study will be documented in the source documents and recorded in the CRF. Medication that was taken prior to V1 and that is still being taken by the subject during the study, will be

considered as a concomitant medication. This applies to both prescription and over-the-counter products.

Records of medication taken include the drug name (preferably both generic and trade name), route of administration (*e.g.*, oral, intravenous), total daily dose/unit (*e.g.*, expressed in mg, mL, or IU), indication, the frequency, the start and (if applicable) the stop date (day, month and year).

7.3.3 Physical Examination

A physical examination will be conducted and the assessment recorded. A full physical examination will include review of general appearance, skin, head, eyes, ears, nose and throat, thyroid gland, chest, lungs, back, abdomen, dentition, cardiovascular, gastrointestinal, musculoskeletal, and neurological systems.

The physical examination is to be conducted by the Investigator or designated fully trained representative and assessed as normal, abnormal – not clinically significant or abnormal – clinically significant.

7.3.4 Body Height and Body Weight, BMI, Waist and Hip Circumferences

Body weight, height, and waist and hip circumferences will be measured. The same scale should be used for all assessments. Height will be expressed in meters (to the nearest centimeter (cm)), and weight in kilograms (to the nearest 0.1 kilogram) in underwear and without shoes.

Body mass index (BMI) will be calculated from the body weight and height using the following formula:

$$\text{BMI} = \frac{\text{weight in kilograms}}{\text{height in meters}^2} \quad (\text{kg/m}^2)$$

Waist-to-hip ratio (WHR) will be calculated by dividing the waist measurement in cm (to the nearest cm) by the hip measurement in cm (to the nearest cm).

7.3.5 Vital Signs

Systolic and diastolic blood pressure, pulse rate and respiratory rate, and body temperature will be measured. Body temperature measurements should be performed first and precede any other study assessment. In case of an elevated temperature, it is the Investigator's decision to determine any further actions according to local practice and their medical judgment. Systolic

and diastolic blood pressure, pulse rate and respiratory rate will be made after the subject has rested for at least 5 minutes in a supine position.

7.3.6 Electrocardiogram

Electrocardiogram (ECG) recording will be performed as per the site's local practice. A standard 12-lead ECG will be recorded after the subject has rested for at least 10 minutes in a supine position. ECG should be performed before spirometry.

The following parameters will be documented: heart rate, PR interval, QRS interval, QT interval, and QT interval corrected by the ECG machine according to Bazett's formula. Every ECG must be assessed as normal, abnormal – clinically not relevant, or abnormal – clinically relevant. A diagnosis must be provided on the CRF for all ECGs assessed as abnormal – clinically relevant. All ECG printouts will be interpreted by a qualified physician. Any printouts of ECGs on thermo-sensitive paper must be photocopied and stapled together for inclusion in the source documents and signed by Investigator.

7.3.7 Lung Function Tests

All personnel performing spirometry testing must have the appropriate training. Quality control measures should be available and be properly documented. The subject will be at rest for at least 15 minutes prior to spirometry testing. All spirometry maneuvers will be recorded with the subject in a sitting position. All spirometry testing must be performed at least 1 hour after smoking or using THS (if applicable) and at least 2 hours after eating. Also, spirometry should be done after vital signs and ECG.

All lung function tests will be managed by a central provider, including the provision of equipment and Investigator spirometry testing site manual. This site manual will include information on equipment, procedures, subject instructions and precautions. All lung function data will be reviewed by blinded over-readers and the acceptability of the overall sessions and individual tests will be provided to the Investigator. At the end of the visit the certified study site staff will be required to send the lung function data to the central provider.

The investigator will assess eligibility of the subjects (exclusion criteria no 8 and no 9) before getting the results from the Spirometry Over-Read Report.

The spirometry test will be performed on a computerized spirometry system, such as Vitalograph® Compact 6600 (Vitalograph; Ennis, Ireland) or similar, in accordance with the 2005 guideline of the American Thoracic Society (ATS)/European Respiratory Society (ERS) Joint Task Force on the standardization of spirometry (69). Spirometry predicted values will be standardized to the ERS Global Lung Function Initiative predictive set (70).

The spirometry tests will include the recording of FEV_1, FVC, FEV_1/FVC ratio and $FEF_{25-75\%}$. All spirometry tests will be performed as described in the Investigator spirometry testing site manual.

Pre- and post-BD spirometry assessments will be performed. Each assessment requires at least 3 valid spirometry tests. The ratio of FEV_1/FVC will be calculated from the highest acceptable FEV_1 and the highest acceptable FVC, respectively.

The results from FEV_1 and the ratio of FEV_1/FVC will be used as eligibility criteria (to assess COPD and asthma conditions) and to address spirometry-related objectives. The investigator will assess eligibility of the subjects (exclusion criteria no 8 and no 9) before getting the results from the Spirometry Over-Read Report. All post-BD spirometry testing will be performed 15-30 minutes post administration of around 400 µg of salbutamol/albuterol (usually equivalent to 4 puffs assuming 100 µg/puff). The time of salbutamol/albuterol inhalation and time of spirometry assessment will be recorded in the source document and CRF. More details will be provided in the Investigator spirometry testing site manual.

7.4 Biomarker Assessment

All bioanalytical assays and laboratory assessments will be carried out using validated methods (sections 7.5 and 7.6). The bioanalytical methods used will be documented in the respective bioanalytical plans/reports.

7.4.1 Biomarkers of Potential Harm

- In blood:

Blood samples will be drawn according to the sample handling manual and laboratory manual in order to measure:

- In serum: TC, TG, HDL-C, LDL-C, ox-LDL, hs-CRP, sICAM-1,
- In plasma: MPO, fibrinogen, IL-6, TNF-α, HCY, suPAR, MDA,
- In blood: WBC, HbA1c.

- In urine:

Spot urine will be collected for analysis of 8-epi-$PGF_{2\alpha}$, 11-DTX-B_2. Creatinine will be measured for normalization of these endpoints.

7.4.2 Biomarkers of Exposure

7.4.2.1 Biomarker of Exposure to CO

CO Breath Test: CO in exhaled breath will be measured using the Vitalograph BreathCO™ device, or similar. The results will be used to assess eligibility. COHb will be measured in blood.

7.4.2.2 Biomarkers of Exposure to HPHCs in Urine

Spot urine will be collected for analysis of NEQ, 2CyEMA, total NNAL.

7.4.3 Creatinine

Creatinine will be measured for normalization of urinary BoExp (total NNAL, NEQ and 2CyEMA) and urinary BoPH (11-DTX-B_2 and 8-epi-$PGF_{2\alpha}$).

7.5 Laboratory Assessments

7.5.1 Urine Drug Screening

A urine drug screen dipstick (amphetamines, barbiturates, benzodiazepines, cannabinoids, cocaine, and opiates) will be performed and used as eligibility criteria.

7.5.2 Urine Cotinine Test

A cotinine test dipstick with a threshold of ≥ 200 ng/mL will be performed to confirm subject's smoking status or THS use status. Another cotinine test, with a threshold of < 100 ng/mL, will be performed to confirm subject's smoking abstinence among former cigarette smokers.

7.5.3 Urine Pregnancy Test

All female subjects will perform a pregnancy test dipstick. Female subjects with a positive pregnancy test will not be enrolled and will be considered as screen failures. In case of any positive urine pregnancy test, the Investigator will inform the subject about the risks associated with smoking or using THS during pregnancy.

All pregnancies detected during the study must be handled as described in section 8.4.

7.5.4 Alcohol Test

A breath alcohol test will be performed and used as eligibility criteria.

7.6 Sample Handling, Storage, and Shipment

All blood and urine samples for biomarker assessment will be tested at the central laboratory/bioanalytical laboratories. The urine drug screen, pregnancy and cotinine dipstick tests will be done by personnel at the study sites.

The bioanalytical lab(s) will be listed in the laboratory manual.

Detailed procedures for collection and handling of samples are described in the sample handling manual and laboratory manual. All samples that were planned for analysis will be destroyed post database lock or post finalization of the bioanalytical reports depending on which one is coming last as described in the laboratory manual.

7.6.1 Blood Samples

Blood samples will be collected by qualified and trained site personnel following the site's standard procedures. The maximal total volume of blood drawn for each subject will be around 48 mL. Additional blood samples (up to 12 mL) will be taken for long term storage and genomic analysis for subjects who consent to these optional procedures (section 7.6.3)

7.6.2 Urine Samples

Spot urine samples will be used for the urine cotinine screening test, urine drug screening, urine pregnancy test and the assessment of urine BoExp, BoPH, creatinine. If enough urine could be collected at screening, the same spot urine will be used for BoPH after enrollment. Otherwise, another spot urine collection will be performed later after enrollment, if possible. Urine samples that might have been collected for subjects who are not enrolled should be discarded.

7.6.3 Long-Term Storage of Whole Blood, Serum and Plasma

If subject gives consent for sample biobanking for long-term storage and analysis for future biomedical research and/or for genomic research, additional samples of whole blood/serum/plasma will be collected as follows:

- Whole blood: 5-6 mL will be collected for future genomic analysis
- Serum/plasma: 5-6 mL of whole blood will be collected to obtain samples of serum and plasma for long-term storage and analysis (biobanking)

Results from these analyses will not be included in the CSR but in a separate report at a later date.

The samples intended for sample biobanking will be kept frozen and shipped to a central storage facility. Samples will be stored for a maximum of 10 years after the signature of the

final study report, unless local country regulations require shorter storage time. If a subject withdraws his/her consent for sample biobanking, the site will inform the Sponsor, who in turn will inform the storage facility about request of sample disposal. The storage facility at which the samples are stored will follow their procedures for destruction of biobanked samples.

7.7 Other Study Procedures

7.7.1 Physiological Measures

7.7.1.1 Augmentation Index and Pulse Wave Velocity

All personnel performing Augmentation Index (AIx) and PWV assessments must have the appropriate training. AIx and carotid/femoral PWV assessments will be conducted at all sites using a SphygmoCor XCEL device or similar according to the manufacturer's instructions.

Prior to measurements, subjects will fast and avoid caffeine for at least 3 hours (described in Section 6.4.2), and will not smoke or use THS for at least 2 hours (section 6.4.1). Subjects will have rested for 10 minutes in a supine position prior to measurements.

7.7.2 Self-Reported Measures

7.7.2.1 Questions on Tobacco/Nicotine-containing Products Use History

Subjects will be asked a set of questions about their tobacco and nicotine-containing products use history. They will self-report their tobacco and nicotine-containing product use. The questions will capture key variables of frequency, quantity, intensity, and duration of both exclusive and multiple product use, current and past use, duration of use, duration of interruption, product characteristics, and patterns of use. This information will be used as characteristics of the study subjects and to assess their eligibility for the study.

7.7.2.2 Lifestyle Questionnaire

The Lifestyle Questionnaire was developed from the Lifestyle Risk Scale (LRS) and the corresponding study published in 2011 (71).

The original Lifestyle questionnaire is composed of 9 items and investigates 6 risk categories: diet (1 item), alcohol intake (1 item), physical activity (2 items), sleep deficit (1 item), exposure to passive smoking (1 item), smoking (1 item) and Body Mass Index (2 items). A modified version of the instrument will be used in the study: four items related to diet, smoking and BMI will be excluded as these topics are already covered: diet by the Qualitative Food Frequency Questionnaire, smoking by the Product Use History Questionnaire, and BMI, by a separate assessment. In addition, the item related to passive smoking will be modified in order to adapt

it to all study groups. The study specific version will consist of 5 items. The item scaling is dichotomous for the exposure of passive smoking item, the numerical type of response is applied for the rest of the items. The subject answers according to her/his habits. The total score of the study specific version is ranging from 0 to 11.5. Higher score indicates higher lifestyle behavior-related health risk. Item 5 in the study specific version of the questionnaire is not included in the global score but gives an indication on passive smoking. This questionnaire will be administered to all subjects for assessment of individual characteristics

7.7.2.3 Questions on Socioeconomic Indicators

The questions on socioeconomic indicators cover 2 socioeconomic metrics, namely perceived financial wellbeing and educational level. Respondents will be asked to answer 2 questions about the perceived financial wellbeing of their household, and their educational level. Country-specific categories will be provided as response options for the question about educational level (72, 73). For the analysis of the question on educational level, the original variables will be harmonized to regional categories. The 6 response options of the perceived financial wellbeing will be converted into three groups (74). This questionnaire will be administered to all subjects for assessment of individual characteristics.

7.7.2.4 Qualitative Food Frequency Questionnaire

The Qualitative Food Frequency Questionnaire was developed by Dehghan et al. (75), and it is a generic questionnaire that can be used in multiple countries despite regional differences in intake of a specific food item within a category. It contains all the main food groups, i.e., dairy, meat, fish, fruits and vegetables, and a few food items that are culture dependent such as tofu and soy sauce.

The Qualitative Food Frequency Questionnaire consists of 20 items, where each item represents a different food item. The questionnaire uses the last 12 months as recall period, and provides response options to indicate the number of times a specific food item was consumed per day, per week, or per month. The frequency of consumption is used for the scoring system. For data analysis, all frequencies of consumption are converted to times per day (for example, a response of 3 servings per week is converted to 0.43 servings per day). This questionnaire will be administered to all subjects for assessment of individual characteristics.

7.7.2.5 ABOUT–Perceived Risk

As part of the ABOUT™ Toolbox (76), the ABOUT–Perceived Risk (77) was developed as a self-report measure designed to assess the perceived risks associated with the use of tobacco and nicotine-containing products (TNPs), including cigarettes, nicotine replacement therapy products (NRTs), and alternatives to cigarettes. The ABOUT–Perceived Risk can also be used to assess the perceived risks associated with the cessation or the past use of cigarettes. The

measure has been developed to assess 2 sets of perceptions: the perceived risks to the individual respondent (personal risk) and the perceived risks to users of the product in general (general risk). The personal risk and the general risk versions of the measure share the same items and response options; only the opening stems differ so that they orient respondents toward consideration of their own risk (personal risk) or the risks to the average user of the product (general risk).

The ABOUT–Perceived Risk consists of 3 domains/scales: Perceived Health Risk scale, Perceived Addiction Risk scale, and 2 single items on Perceived Harm to Others. For this study, the 9-item short version of the Perceived Health Risk scale will be used in order to reduce response burden and since it is applicable for assessing group means rather than individual measures.

Responses are measured on a 5-point Likert-like scale (0=no risk; 1=low risk; 2=moderate risk; 3=high risk; 4=very high risk). "I don't know" response option is also proposed for each item and scoring provides a summary measure for the 9-item short version of Perceived Health Risk scale. The subject responses to the scale's items are transformed through the conversion tables to provide a total scale score that ranges from 0 (no perceived risk) to 100 (very high perceived risk).

The questionnaire will be administered to all subjects as follows:

- THS users (to assess perceived personal risk of THS as current users of the product and perceived personal risk of cigarettes as former cigarette smokers)
- Cigarette smokers (to assess perceived personal risk of cigarettes as current cigarette smokers and perceived general risk of THS)
- Former cigarette smokers (to assess perceived personal risk of cigarettes as former cigarette smokers and perceived general risk of THS).

7.7.2.6 ABOUT–Dependence

The ABOUT–Dependence is part of the ABOUT™ Toolbox (76), and was developed to provide a measure of perceived psychological dependence associated with the use of different TNPs and across exclusive and multiple TNPs users (77, 78). The measure has been validated in a diverse population of TNP users which included exclusive and multiple TNP users across different products such as cigarettes, electronic nicotine delivery devices (e.g., e-cigarettes), smokeless tobacco, cigars/cigarillos, waterpipes, pipes, and nicotine replacement therapies (NRTs). Inclusion of the ABOUT–Dependence in this study will provide further data for validation in users of heated tobacco products such as THS.

The 12-item measure consists of 3 main perceived dependence domains: Extent of use (2 items) referring to the timing of use (time to first product use, time from last product use to going to bed), Signs and symptoms (5 items) which captures the feelings and experience of the

symptoms of perceived dependence, and Behavioral impact (5 items) which captures the behavioral aspects of perceived dependence and impact on daily activities. Responses are measured on 5 to 6-point Likert Scale to indicate increasing severity. The measure can be scored and interpreted using separate scores for each domain or a total composite score from all the 12-items across the 3 domains. Subject raw scores are transformed using conversion tables to provide a total measure score (ranging from 0 (no perceived dependence) to 100 (very high perceived dependence). This questionnaire will be administered for assessment and comparison of self-reported perceived dependence in subjects in the current THS user and current cigarette smoker groups.

7.7.2.7 ABOUT–Health and Functioning

The ABOUT–Health and Functioning is part of the ABOUT™ Toolbox (76), and was developed to provide a measure of perceived health and functioning status associated with the use of different TNPs and with switching to RRPs (79). The measure has been developed considering a diverse population of TNP users which included exclusive and dual TNP users across different products (cigarettes, e-cigarettes, heated tobacco products, and nicotine replacement therapies (NRTs)). The measure consists of 10 main domains/scales of health and daily life functioning (appearance, oral health, throat and voice, coughing, breathing and lung health, breathing difficulty, general health, working life, social functioning, well-being, and bother with smell). The coughing, breathing and lung health, breathing difficulty, and general health domains will be administered in this study. Responses are measured on 6-7-point Likert Scale to indicate severity, frequency, rating, or perceived change. The measure can be scored and interpreted using separate scores for each domains/scales. Subject raw scores are transformed using conversion tables to provide a total measure score for a domain (ranging from 0 to 100). This questionnaire will be administered for assessment and comparison of self-reported perceived health and functioning status in subjects in the current THS user and current cigarette smoker groups.

Symptoms or worsening of symptoms documented in the ABOUT–Health and Functioning questionnaire do not need to be documented as additional AEs because this questionnaire will be analyzed as part of the final report. However, it is at the discretion of the PI(s) or designee(s) to decide whether to document such symptoms as additional AEs. The main source for AE collection will be the face-to-face interview between the subject and study site staff, using open, non-directive questions, as described in section 8.2.1.

7.7.2.8 36-Item Short Form Health Survey (SF-36)

The SF-36 was developed to measure generic health concepts relevant across age, disease, and treatment groups (80). It consists of 36 items, covering the following domains: Physical Functioning (PF) (10 items), Role-Physical (RP) (4 items), Bodily Pain (BP) (2 items), General Health (GH) (5 items), Vitality (VT) (4 items), Social Functioning (SF) (2 items), Role-

Emotional (RE) (3 items), Mental Health (MH) (5 items), and an additional item on Reported Health Transition (perceived change in health).

The SF-36 was designed for use in clinical practice and research, health policy evaluations, and general population surveys and has shown robust measurement properties in terms of reliability, validity, and ability to detect change.

The SF-36 uses dichotomous (Yes/No) and 5 to 6-point Likert Scales as response options. The SF-36 provides norm-based scores by domains and Physical Component Summary (PCS) and Mental Component Summary (MCS) scores can also be calculated. A higher score on the SF-36 indicate better health status. This questionnaire will be administered to all subjects for assessment of self-reported health perceptions

8 ADVERSE EVENTS

8.1 Definitions

8.1.1 Adverse Events

An adverse event is defined as any health-related event which is adverse or unfavorable and which either starts after ICF signature or represents a worsening of a health–related condition that existed at the time of that signature. Careful medical judgment is required to establish whether a clinical finding (including an abnormal test result) is a true AE or just a manifestation of a preexisting health–related condition. An AE may or may not have a causal relationship with the study procedures.

8.1.2 Serious Adverse Events

A serious adverse event (SAE) is defined as an AE that:

- Results in death,
- Is life-threatening,
- Requires inpatient hospitalization or prolongation of existing hospitalization,
- Results in persistent or significant disability/incapacity, or
- Is a congenital anomaly/birth defect.

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered an SAE when, based on appropriate medical judgment, they may jeopardize the subject or the subject may require medical or surgical intervention to prevent one of the outcomes listed in the above definitions.

"Life-threatening" means that the subject was at immediate risk of death from the event. It might have caused death if it had occurred in a more serious form.

8.1.3 Conditions Existent Before the Start of the Period of Collection (ICF Signature)

Clinical conditions that existed before the start of the period of collection of AEs and still ongoing at Visit 1 (concomitant disease), and whose severity or frequency remained unchanged after that point, should not be considered AEs and should not be captured as such. This includes medical therapies or surgical interventions that had been planned before the start of the period of collection regardless of involving admissions to hospital, if the medical condition to be addressed did not get worse after the start of the collection period. Otherwise, any medical

condition that existed before the start of the period of collection and still ongoing at Visit 1 (concomitant disease) and whose severity or frequency increased after that point is to be captured as an AE or SAE, depending on the seriousness criteria met.

8.2 Assessment of Adverse Events

The Investigator is responsible for obtaining, assessing, and documenting all AEs during the study.

8.2.1 Collection of Information

Any non-serious AE occurrence during the study must be documented in the subject's medical records in accordance with the Investigator's normal clinical practice and on the AE page of the CRF. SAEs that occur during the study must be documented in the subject's medical record, on the AE CRF, and on the SAE form.

AEs should be collected mainly via face-to-face interview using open, non-directive questions from the investigator (e.g., "How have you been feeling since you were last asked?").

Information recorded will include: verbatim description of the AE/SAE, start and stop dates, seriousness, severity (intensity), action taken (e.g., whether or not the AE/SAE led to the subject's discontinuation from the study), and outcome (e.g., resolved, stabilized).

Information to be recorded about an AE/SAE should include, whenever possible, onset and resolution dates and times, circumstances leading up to the event, clinical elements such as clinical course, specific vital signs and test results that may explain the pathophysiology of the event, as well as alternative explanations to its occurrence.

Whenever a medically meaningful diagnosis is available to comprise a set of reported signs and/or symptoms, it should be preferentially provided as the AE or SAE term, rather than the individual signs and/or symptoms. Otherwise, each one of those signs and/or symptoms should be reported separately as event terms.

8.2.2 Period of Collection

AEs (including SAEs) will be collected from the time of ICF signature until the individual EOS for each subject.

Any AEs which occur during Visit 1 will be captured by the study site staff and assessed by the Investigator in order to establish relationship to study procedures.

After Discharge at Visit 1, the subject will enter a 1-day FU Period during which AEs reported by the subjects will be collected. Any non-serious AE that is ongoing at the time of discharge will be followed-up by the Investigator during the FU period until it has been resolved, stabilized (i.e., no worsening of the condition), or until subject is lost to follow up.

Any AEs or SAEs that are ongoing at the end of the FU Period will be managed as described in section 8.2.5.

8.2.3 Intensity of Adverse Event

The Investigator must assess the intensity of each reported AE according to the following grading scale:

Mild: Easily tolerated, not interfering with normal daily activity.

Moderate: Interferes with normal daily activity, but the subject is still able to function.

Severe: Incapacitating and requiring medical intervention.

8.2.4 Relationship to Study Procedures

The Investigator must assess the causal relationship between the study procedures and each of the reported AEs, using the classification system and the criteria described below:

Not related: The temporal relationship of the clinical event to study procedures makes a causal relationship unlikely, or, concomitant medication, therapeutic interventions, or underlying conditions provide a sufficient explanation for the observed event.

Related: The temporal relationship of the clinical event to study procedures makes a causal relationship possible, and concomitant medication, therapeutic interventions, or underlying conditions do not provide a sufficient explanation for the observed event.

8.2.5 Follow-up of Non-serious and Serious Adverse Events

Any non-serious AE that is ongoing at the time of Discharge will be followed-up by the Investigator during the FU Period until it has been resolved, stabilized (i.e., no worsening of the condition), or an acceptable explanation has been found (e.g., a chronic condition). The follow-up of the ongoing AEs will be done via a phone call performed at the end of the FU Period. In case they cannot be reached at the initial call, 2 additional attempts on the next 2 consecutive days will be made. At the end of the FU Period, all ongoing non-serious AEs will have the outcome documented as “unknown” and no further follow-up information will be sought on them by the Investigator. At that point, the Investigator will assess whether the subject should be referred to his/her General Practitioner to have their ongoing AEs addressed accordingly.

All SAE will be followed up by the Investigator, despite their continuation after the end of the FU Period, until their resolution, stabilization (i.e., no worsening of the condition), or an acceptable explanation has been found (e.g., a chronic condition). In case the subject cannot

be reached for additional information related to SAE(s), a total 3 attempts should be performed before the subject will be declared as lost to follow-up.

8.3 Reporting and Follow-Up of Serious Adverse Events

Any SAE observed during the period of collection in this study must be reported by that party within 24 hours of first awareness to Sponsor, via email, having the SAE form attached as detailed in the Safety Management Plan (SMP).

As further information regarding an already reported SAE becomes available to any of the parties involved in this study, such follow-up information should be reported on a new SAE report form, marked as a follow-up report and submitted to the Sponsor according to the same timelines as described above. The follow-up SAE report form must include the minimum information required for form completion and only changed/new information needs to be specified. Information provided in the follow-up SAE report form supersedes any information that was initially reported.

The SAE report form to be used in this study is provided as a separate document. All SAEs will also be recorded on the relevant CRF page, in addition to the SAE report form.

The Investigator is responsible for submitting the relevant reports of SAEs that occur during the study to the local IEC, according to local regulations and in accordance with the respective safety management plan (SMP).

8.4 Pregnancies

Pregnancies detected at V1 during the screening phase will be considered as a screen failure. No pregnancy form will be filled; however, the pregnancy must be captured in the reason for screen failure CRF page. Smoking or THS use cessation advice will be provided to subjects and subjects will be referred to health care facility/health care provider for pregnancy follow-up.

9 STUDY ACTIVITIES

A detailed schedule of assessments can be found in Appendix A.

9.1 Screening, Enrollment and Assessment Visit: V1

First, the main ICF along with study information should be given to the subject. When/if the main ICF is signed, dated and timed, the screening procedures can be performed. All eligibility criteria will be checked, in the order deemed most practical. The point of time of each screening procedure must be recorded in the source document. Subjects who meet all eligibility criteria will be enrolled. Subjects who do not meet the previous criteria will be considered as screen failures.

Table shows the assessments that will be performed at the screening/enrollment/assessment visit (Visit 1).

Some assessments need to be performed before enrollment, some after enrollment (please refer to Table). Within assessments to be performed before or after enrollment, the sequence of assessments/events is given just for illustration purposes, except if specified in the additional information.

Table 2 Time Schedule – V1

Procedures	Additional Information
Prior to enrollment	
ICF(s) signature	To be performed before any study procedure. (section 7.1)
Vital signs	Section 7.3.5. Body temperature measurements should be performed first and precede any other study assessment.
Information on the risks of smoking and smoking cessation advice	Section 7.2
Demographic data collection	Section 7.3.1
Prior/concomitant medication	Section 7.3.2
Medical history/concomitant diseases	Section 7.3.2
Questions on tobacco/nicotine-containing products use history	Section 7.7.2.1
Body height, weight and BMI	Section 7.3.4

Procedures	Additional Information
CO breath test	Section 7.4.2.1
Alcohol breath test	Section 7.5.4
U: Spot urine collection for drug screen, pregnancy and cotinine tests.	Pregnancy test only on female subjects (section 7.5.3). There are 2 different cotinine tests: (section 7.5.2) Process urine samples according to laboratory manual/Sample Handling Manual (SHM) (section 7.6).
Physical examination	Section 7.3.3
ECG	Section 7.3.6. Vital signs and ECG should be performed before spirometry per Section 7.3.7.
Spirometry pre- and post-BD	Section 7.3.7 The investigator will assess eligibility of the subjects (exclusion criteria no 8 and no 9) before getting the results from the Spirometry Over-Read Report.
Inclusion/exclusion criteria	All eligibility criteria must be checked.
Enrollment	If all eligibility criteria are met, all the below assessments below to be performed.
After enrollment	
Waist and hips circumference	Section 7.3.4
B: Blood collection for BoPH/BoExp	Process blood samples for BoPH/ analysis according to laboratory manual/SHM.
U: Spot urine collection for BoExp and creatinine samples.	The same spot urine collection will be used for pregnancy (all females), drug screen, cotinine tests, creatinine and BoExp to HPHCs/nicotine.
U: Spot urine collection for BoPH	If enough urine could be collected, the same spot urine will be used for BoPH. Otherwise another spot urine collection will be performed later during the visit.
Lifestyle Questionnaire	Section 7.7.2.2
Qualitative food group frequency questionnaire	Section 7.7.2.4
Questions on socioeconomic indicators	Section 7.7.2.3
ABOUT–Perceived risk	Section 7.7.2.5

Procedures	Additional Information
ABOUT–Dependence	Section 7.7.2.6
ABOUT–Health and Functioning	Section 7.7.2.7
SF-36	Section 7.7.2.8
AIx & PWV	Must be done at least 2 hours after having stopped smoking/using THS and 3 hours after large meal and caffeine-containing food/drinks.
B: Biobanking	If optional consent(s) is/are signed. Section 7.6.3. Process blood samples for biobanking according to laboratory manual/SHM.
AE/SAE recording	Section 8. At any time during the visit.
Discard any samples that might have been collected for subjects who are screen failure.	

Abbreviations:

B = blood; U = urine

9.2 Safety Follow-up Period

After Discharge at Visit 1, the subject will enter a 1-day FU Period during which AEs spontaneously reported by the subjects will be collected and ongoing AEs will be followed by site, as described in sections 8.2.2 and 8.2.5.

10 QUALITY CONTROL AND QUALITY ASSURANCE

10.1 Monitoring

The Clinical Research Associate ("Monitor") of the Contract Research Organization (CRO) will be responsible for the monitoring of the study. Monitoring will be performed according to CRO's SOPs and as per the monitoring plan agreed with the Sponsor.

The Investigator shall permit the Monitor to review study data as frequently as considered necessary to ensure that data are being recorded in an adequate manner and that protocol adherence is satisfactorily met.

The Investigator shall access medical records for the Monitor in order that entries in the CRFs may be verified. As part of his/her(their) responsibilities, the Investigator is expected to ensure that the study adheres to GCP principles (81).

An Investigator's meeting will be held prior to the site initiation visit. During this meeting, the general training on the study procedures and specific training on selected procedures will be completed and documented.

Subsequent to the Investigator's meeting, and before the first subject is screened into the study, the site initiation visit will be conducted by the Monitor and, if necessary, together with the Sponsor or its authorized representative. The purpose of the site initiation visit is detailed in the monitoring plan.

During the study, the Monitor will have regular contact with the study site, including interim monitoring visits. The purpose of these visits is described in the monitoring plan. The monitoring is performed using a risk-based approach as described in the monitoring plan (please refer to section 10.2 describing overall study risk management).

Communication by telephone, mail, and e-mail may be used as needed to supplement site visits. The Investigator and study personnel will cooperate with the Monitor, provide all appropriate documentation, and will be available to discuss the study.

The Monitor and the Sponsor's personnel will be available between visits should the Investigator or other staff at the sites need information and advice.

Site visits will be made at regular intervals during the study. The frequency of the monitoring visits will be defined in the monitoring plan agreed with the Sponsor.

The Investigator must be available during the monitoring visit to review the data, resolve any queries and to allow direct access to the subject's records for source data verification.

10.2 Risk Management

According to ICH-GCP E6(R2) Section 5, the Sponsor will implement a system to manage quality throughout all stages of the study process (2). Pursuant to this, a risk management process will be implemented including identification and scoring of risks, identification of critical data and processes as well as the definition of Key Risk Indicators (KRI) and Quality Tolerance Levels (QTL).

This risk management approach will be described in an Integrated Risk and Quality Management Plan with risks described in a Risk Assessment and Categorization Tool (RACT) which will be developed during the set-up phase of the study and reviewed throughout all stages of the study.

In addition, at the end of study, the Sponsor will describe the quality management approach implemented in the study and summarize important deviations from the predefined quality tolerance limits and remedial actions taken in the CSR.

10.3 Training of Staff

A formal meeting (Investigator's meeting) will be conducted prior to site initiation. During this meeting, the Sponsor or its authorized representative will discuss the requirements of the clinical study protocol and related documents and will also provide training on the relevant systems and other study-specific procedures. The activities of this meeting will be described in the monitoring plan.

In addition to the Investigator meeting, the Investigator will ensure that appropriate training relevant to the study is provided to all staff involved in the study, and that any new information relevant to the performance of this study is forwarded in a timely manner to the staff involved. The Investigator will maintain a record of all individuals involved in the study.

10.4 Audits and Inspections

Good Clinical Practice regulations require that there are independent inspections of clinical program activities. Such inspections may be performed at any time before, during and/or after the study.

Authorized representatives of the Sponsor, regulatory agencies and/or an IRB/IEC may perform audits or inspections, including source data verification. The purpose of an audit or inspection is to systematically and independently examine all study-related activities and documents to determine whether these activities were conducted and data were recorded, analyzed, and accurately reported according to the protocol, ICH/GCP guidelines (81), and any applicable regulatory requirements. The Investigator will contact the Sponsor or the authorized representative immediately if contacted by a regulatory agency about an inspection at their site.

The Investigator is responsible for maintaining a comprehensive and accurate filing system of all study-related documentation that will be suitable for inspection at any time by the Sponsor, its authorized representative, and/or regulatory agencies. By signing this protocol, the Investigator understands and agrees to provide access to the necessary documentation and files.

11 DATA MANAGEMENT ACTIVITIES

All data management activities will be described in detail in the Data Management Plan (DMP) and documents specified therein. The electronic systems used to collect subject data, including eCRF and electronic patient reported outcome (ePRO), will be FDA 21 CFR Part 11 compliant.

11.1 Data Capture

11.1.1 Case Report Forms and Study Records

Results from the clinical assessments will be recorded in the source documents by the Investigator and then captured in the CRFs at the study site, except for the biomarkers results, the augmentation index and pulse wave velocity results which will be transferred directly to the clinical database as per the specific Data Transfer Agreement. The subject questionnaires will be entered by the subject directly into an ePRO and automatically integrated into the eCRF. Spirometry results will also be integrated into the eCRF. Trained study personnel will be responsible for capturing the data from the observations, tests and assessments specified in the protocol in the source documents, and then for transferring the data into the eCRF according to the CRF Completion Guidelines.

The Investigator has ultimate responsibility for the collection and reporting of all data related to the clinical study and for ensuring that the data are accurate, authentic/original, legible, timely (contemporaneous), enduring, and available when required. The CRF must be signed by the Investigator to attest that the data contained on the CRF are true and accurate. Any corrections made to source documents and/or CRFs must be clearly recorded, without obscuring the original values and must be accompanied by the date of change, reason for change, and identification of the person making the change. The CRF for each subject will be checked against the source documents at the study site by the Monitor. Instances of missing or unclear data will be discussed with the Investigator for resolution. For the electronic questionnaires, all subject reported questionnaire data will be provided in English. Questionnaires and instructions will be provided in the subject's local language. A CRF will be generated for all subjects who have signed the ICF.

11.1.2 Protocol Deviations

Protocol deviations are defined as deviations from the study procedures as defined in this document, including but not limited to any violation of inclusion/exclusion criteria, assessments not performed or performed outside the scheduled time windows, or use of drugs that are known to affect endpoints (Appendix B). Classification of protocol deviations as minor/major deviations and subsequently major deviations impacting or not the evaluability of the results will be defined in the SAP.

All protocol deviations will be documented in the clinical trial management system (CTMS) or other approved format.

Information from the source documents will represent the primary source of protocol deviations. Information following site monitoring and other manual reviews will be documented in the site visit reports, follow-up letters, audit documentation, or other manual review and will be recorded and tracked in the CTMS or other approved format. Telecommunications and other verbal communications regarding deviations will be considered and handled as important communication, documented and tracked as protocol deviations, as necessary.

Individual entries for protocol deviations that are recorded in the CTMS, or other approved format, following site monitoring and other manual reviews will be reviewed against the individual data points in the CRF database. The overall procedure for managing protocol deviations is described in the SOPs and/or agreed upon procedure of the CRO data management team. All deviations will be reviewed periodically, as determined at study start, to identify trends to improve monitoring and/or potential impact on the statistical analysis.

11.2 Data Handling

All study data will be managed by the data management team at the CRO responsible for this study. The overall procedures for quality assurance of clinical study data are described in the SOPs of the CRO data management team. The data management team at the CRO will prepare a DMP, to be reviewed and approved by the Sponsor, prior to the start of data entry. This document will describe, in details, the data management-related procedures and processes.

Data of all subjects who sign the ICF, including screen failures, will be captured in the source documents. For screen failure, only the following data will be captured in the CRF: ICF date and time, demographics, date and reason for screen failure and AE if any. Data from the online pre-screening will not be included in the CRF.

All data collected during the study, except data collected as part of the online pre-screening, is declared property of the Sponsor, irrespective of the location of the database and the data management CRO.

11.2.1 Data Validation

The data will be validated as defined in the DMP and Data Validation Specifications. Discrepancies will be reported and handled as defined in DMP and Data Validation Specifications.

Data queries will be raised for discrepant or missing data. All changes to data in the eCRF will be captured in the database with a comprehensive audit trail.

11.2.2 Coding

AEs, medical/surgical history, and prior/concomitant medication will be classified according to the terminology of the latest version of the following Dictionaries, at time of coding the first entry:

Medical history:	Medical Dictionary for Regulatory Activities (MedDRA®)
Adverse events / Procedures:	MedDRA®
Medications:	WHODrug Global

11.2.3 Database Lock

When all outstanding Data Management issues have been resolved and all validation, quality review, and cleaning activities are complete, the database or selected data is/are declared soft locked as defined in the DMP. Access to the soft-locked database to change data or to change selected data at this time is limited.

After the data is reviewed by the Sponsor, resolution of all raised queries and QC of the changed data, database, or selected data upon Sponsor approval as applicable, is declared locked.

Any changes to the database after that time can only be made by written agreement between the Sponsor and the Data Management and Statistical Teams at the CRO and follow the database unlock process defined in the DMP. Any of those changes must be documented in the database log file.

After study completion, the study database will be transferred to the Sponsor in the format specified in the DMP and compliant with the Clinical Data Interchange Standards Consortium (CDISC) Study Data Tabulation Model (SDTM).

12 PLANNED STATISTICAL METHODS

12.1 General Considerations

Full details of the statistical analysis are given in a Statistical Analysis Plan (SAP). Any changes to the planned statistical methods will be documented in the clinical study report. The statistical evaluation will be performed using SAS®, version 9.2 or later.

12.1.1 Stratification Criteria

To further describe the endpoints, the following stratification criteria will be used:

1. Age (< 40, 40-50, > 50 years old),
2. Sex (Female, Male),
3. Region (Asia, Europe),
4. Average daily product consumption over the last 2 years as self-reported (smoking 10 to 19 cig/day or using 10 to 19 heatsticks/day vs. > 19 cig/day or > 19 heatsticks/day),
5. Tobacco products use history,
6. Time since quitting/switching.

Further definitions of the strata will be provided in the SAP.

12.1.2 Definitions for Statistical Data Analysis

At the time of the present protocol, no new term is defined. Should there be further definitions for the data analysis, they will be defined in the SAP.

12.1.3 Descriptive Statistics

All data will be presented in listings, ordered by group, subject, and study day unless otherwise specified.

For continuous data, summary statistics will include the number of subjects (n), the number and percent of subjects with missing data, the arithmetic mean, arithmetic standard deviation (SD), median, first and third quartiles, minimum, maximum; for log-normal data the geometric mean and geometric coefficient of variation GCV will be presented instead of arithmetic mean and SD.

For categorical data, frequency counts and percentages will be presented.

12.1.4 Handling of Missing Values and of Values outside the Detection Limits

For laboratory parameters:

- Values below the lower limit of quantification (LLOQ) will be imputed using LLOQ/2. For values above the upper limit of quantification (ULOQ), the ULOQ will be imputed.
- The number of values below LLOQ or above ULOQ will be presented in each summary table. If 50% or more data are below LLOQ or above ULOQ, only the number (%) of value below LLOQ or above ULOQ will be reported in the summaries, together with minimum and maximum of the observed values.

Handling of missing answers to questionnaires will be described in the SAP.

12.1.5 Significance Level for Inferential Analysis

The overall, study-wise, type I error will be preserved at 2.5% 1-sided for both the primary objective endpoints and the key secondary objectives endpoints by:

- Testing the endpoints from the primary objective as "co-primary" at a 2.5% 1-sided test-wise alpha level (2-sided 95% confidence intervals will be reported), and
- Testing the key secondary objectives only if the endpoints used for the evaluation of the primary objective have all reached statistical significance simultaneously and using the Hochberg procedure for adjustment for multiplicity on the marginal p-values with a target of 2.5% 1-sided test-wise alpha level (2-sided confidence intervals will be reported using confidence levels based on the actual alpha level of the Hochberg procedure).

Unless stated otherwise, for other analyses the statistical tests are 2-sided and are conducted at the 5% level, and all quoted confidence intervals are 2-sided 95% confidence intervals.

No test multiplicity adjustment will be made for the other secondary objectives.

12.2 Determination of Sample Size and Power Consideration

The sample size and the power have been determined using 10,000 simulation with SAS, ensuring the type I error will be preserved at 2.5% 1-sided for both the co-primary and the key secondary endpoints (using the Hochberg procedure for adjustment for multiplicity).

Enrolling 300 cigarette smokers and 300 THS users, and assuming 95% of the subject will be in the Modified Per-Protocol analysis set (285 in both groups), the study will provide more than:

- 99% power to demonstrate a beneficial difference on all endpoints used for the evaluation of the primary objective simultaneously (COHb, total NNAL, WBC and 8-epi-$PGF_{2\alpha}$),
- 95% power to demonstrate a beneficial difference on the following endpoints used for the evaluation of key secondary objectives: HDL-C, sICAM-1, 11-DTX-B_2,
- 73% power to demonstrate a functional benefit with the endpoint used for the evaluation of the key secondary objective: FEV_1 %predicted, post-BD,
- 53% power to demonstrate a functional benefit with the endpoint used for the evaluation of the key secondary objective AIx.

The assumptions for the effect size and the variability used in the power and sample size simulations are described in Table . More specifically, the assumptions on:

- 8-epi-$PGF_{2\alpha}$, WBC, total NNAL, HDL-C, sICAM-1, 11-DTX-B_2, FEV_1 %predicted (post-BD) are derived from a heat-not-burn tobacco product (HNBP) post-marketing study (62), where:
  - The effect size between cigarette smokers and THS users has been assumed to be equal to the reported effect size between cigarette smokers and HNBP after an average use of 1.2 years.
  - The related variability has been assumed to be the maximum observed between the comparison of the cigarette smokers to the non-smokers, and the comparison of cigarette smokers to the HNBP users.
- COHb are taken from the ZRHR-ERS-09-US study (40)
- AIx are derived from the Cardiovascular and Metabolic Diseases Etiology Research Center (CMERC) cohort study (82) where:
  - The effect size is defined as 75% of the effect size observed between cigarette smokers and subjects having quit smoking for less than 3 years.
  - The variability has been defined as the maximum variability observed (comparing cigarette smokers to non-smoker) which is similar to the one observed in other studies (83, 84).

Table 3 Description of the effect size and variability assumptions used in the power and sample size simulations.

Objectives	Hypothesis	Endpoints	Study Power	Assumptions	Ref.
Primary Objective	H_1	COHb	99.8%	0.677 ± 3.28 (1)	(40)
		Total NNAL		0.057 ± 22.1 (1)	(62)

		8-epi-$PGF_{2\alpha}$		0.784 ± 3.74 [1]	(62)
		WBC		0.822 ± 2.23 [1,4]	(62)
Key Secondary Objectives	H_2	HDL-C	97.6%	1.139 ± 2.23 [1,4]	(62)
	H_3	sICAM-1	95.8%	0.876 ± 2.39 [1]	(62)
	H_4	11-DTX-B_2	98.2%	0.755 ± 5.37 [1]	(62)
	H_5	AIx	53.2%	-1.2 ± 16 [2]	(82-84)
	H_6	FEV_1 %predicted [3]	73.4%	1.050 ± 1.52 [1,5]	(62)

[1] Geometric Mean Ratio (THS:cigarette) ± Geometric Standard Deviation
[2] Mean Difference (THS – Cigarette) ± Standard Deviation
[3] Post-Bronchodilator
[4] The statistical analysis will be performed on the normal scale instead of the natural log scale. As the power is close to 100% the potential loss of power is expected to be minimal.
[5] The statistical analysis will be performed on the normal scale instead of the natural log scale, since FEV1 %predicted Post-Bronchodilator is likely normally distributed as observed in the ZRHR-ERS-09-US study (40). The actual power is conservative.

12.3 Analysis Sets

12.3.1 Full Analysis Set (FAS)

The Full Analysis Set will be composed of all subjects attending Visit 1.

12.3.2 Modified Per-Protocol Analysis Set (mPP)

The Modified Per-Protocol Analysis Set will be a subset of the FAS and will exclude:

- Subjects with major deviations impacting the evaluability of the primary objective,
- Subjects not belonging to a complete triplet (with one current smoker, one former smoker, and one THS user) used for the matching as described in section 4.1.

12.4 Primary Objective Analyses

12.4.1 Primary Estimand

The primary estimand of the primary objective (section 3.1) is defined by the following components:

- **Product Use Under Evaluation**: The regular and predominant use of THS for at least 2 years as compared to the regular and predominant use of cigarettes. The use of other tobacco or nicotine product being very limited or inexistent (further defined per relevant inclusion criteria in section 5.1.1)

- **Target Population**: The target population is defined as the Modified Per-Protocol Analysis Set (section 12.3.2) and the relevant eligibility criteria (sections 5.1.1 and 5.1.2), notably defined by healthy adult subjects, 30 – 60 years old who have a significant smoking history over the 10 last years (see section 5.1.1).
- **Variables of interest**:
  - COHb in blood,
  - Total NNAL in urine,
  - WBC in blood,
  - 8-epi-$PGF_{2\alpha}$ in urine (expressed as concentration adjusted to creatinine),
- **Intercurrent Events** (concurrent intercurrent event will be treated in the order of appearance in the following list):
  - Non-adherence to product use is handled through inclusion criteria as defined in section 5.1.1. and are notably based on self-reported product use, cotinine test, and CO breath test.
  - Concomitant medications, physical conditions and diseases that could potentially impact the level of the variable of interest are handled through exclusion criteria as defined in section 5.1.2.
  - Discontinuations and Missing Data are not expected to be associated with groups or endpoints, and are unlikely given the 2 days duration of this study (including the safety follow-up). Discontinuation effect and missing data will be ignored.
- **Population-Level Summary**:
  - The mean difference of WBC in blood at Visit 1 between THS user and cigarette smokers.
  - The geometric mean ratio of COHb in blood, total NNAL in urine and 8-epi-$PGF_{2\alpha}$ in urine (expressed as concentration adjusted to creatinine) at Day 1, between THS user and cigarette smokers.

12.4.2 Baseline Comparability

Not applicable.

12.4.3 Descriptive Summary

Endpoints related to the primary objective will be summarized at Visit 1 for the FAS and mPP as described in section 12.1.3. Further descriptive statistical summaries will be performed by stratification criteria as described in section 12.1.1.

12.4.4 Primary Estimand Analyses

Primary Hypothesis:

The primary hypothesis (H_1) to be tested is:

- The geometric mean ratio (THS users/current smokers) of COHb in blood is less than 1, and
- The geometric mean ratio (THS users/current smokers) of total NNAL in urine less than 1, and
- The mean difference (THS users - current smokers) of WBC is less than 0, and
- The geometric mean ratio (THS users/current smokers) of 8-epi-$PGF_{2\alpha}$ is less than 1 (in urine, expressed as concentration adjusted to creatinine).

The COHb, total NNAL, WBC and 8-epi-$PGF_{2\alpha}$ endpoints will be tested at 1-sided 2.5% test-wise level, using an intersection-union test, maintaining a type I error level of 2.5% 1-sided for the primary analysis.

Evaluation Criterion:

The study will be declared successful if a beneficial difference is demonstrated on all endpoints simultaneously (COHb, total NNAL, WBC and 8-epi-$PGF_{2\alpha}$) using a one-sided test-wise type I error level of 2.5%.

Missing Data Strategy:

For the main, sensitivity, and supplementary analyses related to the primary estimand of the primary objective, data will not be imputed.

Main Analysis:

The main analysis of the primary objective will be done in the mPP.

Analysis will be performed using generalized linear mixed models:

- Adjusting for:
    - The matching variables (as described in section 4.1), and

- The interaction between product use group and the natural log time (in years) in the product use group (time since quitting/switching/smoking),

- Including study site as a random effect.

Modeling assumptions will be evaluated and model fit assessed by the analysis of residuals (model diagnostics) and by comparing the values predicted versus the observed endpoint values (calibration).

Sensitivity Analyses:

Sensitivity analyses will be performed as described for the main analysis at the exception that:

- WBC will be additionally analyzed on the natural log scale for the main analysis and all sensitivity and supplementary analysis.

- Doubly robust estimator will be used in the FAS and mPP, implementing:
  - Propensity score methods weighting on the odds to be in the THS group, to ensure balanced distribution over the matching variables and also over additional potential confounders, that will be defined in the Statistical Analysis Plan,
  - Stepwise regression on the additional potential confounders.
- THS users and the former smokers having 2CyEMA in urine adjusted to creatinine greater than 47 ng/mg will be excluded and the analysis will be performed as described for the doubly robust estimator in the FAS and mPP. This threshold corresponds to the average concentration in smokers using less than 4 cigarettes per day (85).

Supplementary Analyses:

Supplementary analyses will be performed as described for the main and sensitivity analysis at the exception they will compare:

- The former smokers to the current smoker group

Further details will be provided in the Statistical Analysis Plan.

12.5 Secondary Objectives Analyses

12.5.1 Key Secondary Analyses

12.5.1.1 Primary Estimand

The primary estimand for the key secondary objectives (see section 3.2.1) is defined similarly to the primary estimand for the primary objective analysis (see section 12.4.1) with the notable exception that the population-level summary and related variables of interest between THS users and current smokers groups are:

- The mean difference of HDL-C,
- The geometric mean ratio of sICAM-1,
- The geometric mean ratio of 11-DTX-B2,
- The mean difference of AIx,
- The mean difference of FEV_1 %predicted (post-BD).

12.5.1.2 Baseline Comparability

Not applicable.

12.5.1.3 Descriptive Summary

As described for primary analysis in section 12.4.3.

12.5.1.4 Primary Estimand Analysis

Key Secondary Hypotheses:

The key secondary hypotheses (H_2 to H_8) to be tested are:

- H_2: The mean difference (THS users - current smokers) of HDL-C is greater than 0,
- H_3: The geometric mean ratio (THS users/current smokers) of sICAM-1 is less than 1,
- H_4: The geometric mean ratio (THS users/current smokers) of 11-DTX-B_2 is less than 1,
- H_5: The mean difference (THS users - current smokers) of is less than 0,
- H_6: The mean difference (THS users - current smokers) of FEV_1 %predicted, post-BD is greater than 0.

The key secondary hypotheses will be tested only if the primary hypothesis (H_1) reach statistical significance, using the Hochberg procedure for adjustment for multiplicity on the marginal p-values with a target of 2.5% 1-sided test-wise alpha level, maintaining a 1-sided type I error level of 2.5% between and within the primary and the key secondary analyses.

Evaluation Criterion:

The study will be declared to have demonstrated additional benefits for each key secondary objective for which the main analysis of the primary estimand is successful.

Missing Data Strategy:

As described for primary objective analysis in section 12.4.4.

Main Analysis:

As described for primary objective analysis in section 12.4.4.

Sensitivity Analyses:

As described for primary objective analysis in section 12.4.4.

Supplementary Analysis:

As described for primary objective analysis in section 12.4.4.

12.5.2 Other Secondary Analysis

12.5.2.1 Other Secondary Endpoint Analysis Variables

See section 3.2.2 for the definition of other secondary objectives and related endpoints.

The details on derivation rules, and scoring algorithms are provided in the SAP.

12.5.2.2 Baseline Comparability

Not applicable.

12.5.2.3 Descriptive Analyses

Endpoints related to the other secondary objectives will be summarized at Visit 1 for the FAS and mPP as described in section 12.1.3.

12.5.2.4 Inferential Analysis

The differences related to the endpoints of the other secondary objectives will be tested at Visit 1 using the same methodology and populations (mPP and FAS) as described for the main, sensitivity and supplementary analysis of the primary estimand described in section 12.4.4

12.5.3 Safety Analysis

The number and percentage of subjects with AEs and SAEs will be tabulated by the Medical Dictionary for Regulatory Activities (MedDRA, latest version available at the time the coding activity starts) system organ class (SOC) and preferred term (PT) for the FAS. Summaries will also be presented for AEs by relatedness to study procedures and AEs by severity. Tabulations will include both the number of subjects experiencing an event and the number of events, for the overall population.

12.5.4 Additional Analysis

Selected endpoints (HDL-C, WBC, sICAM-1, 11-DTX-B_2, 8-epi-$PGF_{2\alpha}$, COHb (%), FEV_1 %predicted (post-BD), and total NNAL) tested as multiple endpoints from a previous study (42), as well as AIx will be further analyzed as described in section 12.5.2, with the exception that it will be stratified by region (Asia, Europe).

12.6 Exploratory Analysis

12.6.1 Exploratory Endpoint Analysis Variables

See section 3.3 for the definition of exploratory objectives and related endpoints.

The details on derivation rules will be provided in the SAP.

12.6.2 Baseline Comparability

Not applicable

12.6.3 Descriptive Analysis

Endpoints related to the exploratory objectives will be summarized at Visit 1 for the FAS and mPP as described in section 12.1.3.

12.6.4 Inferential Analysis

The differences related to the endpoints of the other secondary objectives will be tested at Visit 1 using the same methodology and populations (mPP and FAS) as described for the main, sensitivity and supplementary analysis of the primary estimand described in section 12.4.4

12.7 Demographics and Characteristics

The following parameters: age, sex, race, weight, height, WHR, BMI, heart rate, blood pressure, HbA1c, socioeconomic indicators, dietary intake, lifestyle, and tobacco/nicotine-containing products use history will be summarized by group and by stratification criteria as described in section 12.1.1 for the FAS and mPP.

Further details will be provided in the SAP.

12.8 Interim Analysis

Not applicable.

13 ADMINISTRATIVE CONSIDERATIONS

13.1 Study Administrative Structure

13.1.1 Sponsor

The list of Sponsor personnel will be provided as a separate document.

13.1.2 List of Investigators and Sites

The list of Investigators and sites will be provided as a separate document.

13.2 Subject Confidentiality

All information obtained during the conduct of the study with respect to the subjects' state of health will be regarded as confidential. A statement to this effect will be included in the information provided to the subject. An agreement to disclose any such information in the framework of the study will be obtained from the subject in writing and signed by the subject, in compliance with all applicable data protection and privacy legislation.

The anonymity of subjects participating in this study will be maintained. Subjects will be identifiable by the Sponsor (or Sponsor's authorized representative) on CRFs and other documents by their subject number/code, sex, and age, but not by name, initial, or any other details relating to identifiable person (e.g., address, social security number, health insurance ID card, medical chart number, etc.). The assignment of a subject number/code for subject identification will be based on the appropriate data protection rules.

Any documents that allow full identification of the subject (e.g., the subject's signed ICF) must be kept confidential by the Investigator. If any document relating to this study shows a subject's name or any other details relating to an identifiable person (e.g., address, social security number, health insurance ID card, medical chart number, etc.), the name or other identifiable details must be obscured before a copy of that document is supplied to the Sponsor or the Sponsor's authorized representative.

13.3 Access to Source Documents

Subjects will be informed that, during as well as after the course of the clinical study, the Sponsor, any authorized representatives of the Sponsor, IRB/IEC, or regulatory authorities may inspect their medical records to verify the information collected, and ensure that all personal information made available for inspection is handled in the strictest confidence and in accordance with applicable data protection and privacy legislation.

The Investigator and all study site staff involved in the study must permit direct access to source data/documents for study related monitoring, audits, IRB/IEC review, and regulatory inspection(s).

13.4 Record Retention

All records of data, source data and source documents (original records or certified copies), in any form (including, but not limited to, written, electronic, magnetic, optical records and scans, and ECGs) that describe or record the methods, conduct, and/or results of the study, the factors affecting the study, and the actions taken will be maintained by the Investigator/study site for the study, as required by ICH GCP (2) and any other applicable local or national regulations.

Essential study documents/records, which individually and collectively permit evaluation of the conduct of a study and the quality of the data produced, are described in Section 8 of the ICH Tripartite Guideline for Good Clinical Practice (2) and in Article 41 of Ministerial Ordinance on GCP (Ordinance of the Ministry of Health and Welfare No. 28 of March 27, 1997 (as last amended by the Ordinance of Ministry of Health, Labor and Welfare No. 161 of December 28, 2012) (4).

Unless other countries law requires archiving for a longer period, the Sponsor and the investigator shall archive the essential documents for at least 25 years after the end of the clinical trial. The medical files of subjects shall be archived in accordance with national law.

Examples of essential records/documents include, but are not limited to:

- Signed informed consent documents for all subjects and master ICF.
- Subject identification code list, screening log, and enrollment log (if applicable).
- Record of all communications between the Investigator and the IRB/IEC, composition of the IRB/IEC.
- Record of all communications/contact between the Investigator, Sponsor, and its authorized representatives.
- List of sub-Investigators and other appropriately qualified persons to whom the Investigator has delegated significant study-related duties, together with their roles in the study, curricula vitae, and their signatures.
- Investigator logs.
- CRFs, study specific questionnaires (and associated data/scoring), subject diaries.
- AE reports and details of follow-up investigations, details of concomitant medication.
- All other source documents (e.g., ECGs, consultation reports, physical examination, laboratory records) or any electronically captured study source data.

- Clinical laboratory reports, laboratory normal ranges.
- Original medical/hospital records, if applicable (the medical files of study subjects must be retained in accordance with local legislation and in accordance with the maximum period of time permitted by the hospital or study site).
- Record of any body fluids or tissue samples collected and retained.
- Information regarding subjects' discontinuation and any follow-up.

The Investigator/study site must take measures to prevent accidental or premature destruction of these documents.

If the Investigator wishes to assign the study records to another party or move them to another location, the Sponsor must be notified in advance.

The Investigator must obtain written approval from the Sponsor before destruction of any records. Normally, these records will be held in the archives of the Investigator. If an Investigator is unable to meet the archiving obligation, he/she must ask the Sponsor for permission to make alternative arrangements. Details of these arrangements must be documented.

13.5 Clinical Study Report

The Sponsor will ensure that a CSR for this study is prepared regardless of whether the study is completed or prematurely terminated.

The CSR will be written based on standards of the ICH Guideline for the structure and content of clinical study reports (ICH E3) (86). In certain circumstances, an abbreviated CSR may be acceptable. CSR will be submitted to the IRB/IEC in compliance with local requirements.

13.6 Financial Disclosure

Investigator is required to disclose financial information to the Sponsor. In addition, the Investigators must commit to the Sponsor to promptly update this information if any relevant changes occur during the course of the investigation and for one year following the completion of the study.

13.7 Publication and Disclosure Policy

This document contains information that is confidential and proprietary to the Sponsor. This information is being provided solely for the purpose of evaluation and/or conducting this clinical study for the Sponsor. The content of this document may only be disclosed to study personnel, IRB/IEC, or duly authorized representatives of regulatory agencies for the purpose

mentioned above under the condition that confidentiality is maintained. The contents of this document may not be used in any other clinical study, disclosed to any other person or entity without the prior written permission of the Sponsor. The foregoing shall not apply to disclosure required by any regulations; however, the Investigator will give prompt notice to the Sponsor prior to any such disclosure.

The Sponsor plans to disclose details of the study protocol in a web-based, publicly available, clinical trial registry database (e.g., ClinicalTrials.gov).

13.8 Insurance

The Sponsor is responsible for AEs and health damage to the subjects when they are associated with study procedures which are used during the study, except for AEs and health damage caused to the subjects by a negligent act or an intentional misconduct and/or significant deviation to the protocol by the Investigator or the clinical study site or the subjects. The Sponsor has taken out insurance to cover any bodily injury and property damage caused by the operations carried out by the insured.

14 REFERENCE LIST

1. Deveci SE, Deveci F, Acik Y, Ozan AT. The measurement of exhaled carbon monoxide in healthy smokers and non-smokers. Respiratory medicine. 2004;98(6):551-6. Epub 2004/06/12. PubMed PMID: 15191041.

2. ICH E6 (R2). Integrated addendum to ICH E6 (R1): guideline for good clinical practice - Current *Step 4* version dated 9 November 2016. 2016;Available from: https://database.ich.org/sites/default/files/E6_R2_Addendum.pdf (Accessed on 17 January 2020).

3. World Medical Association (WMA). Declaration of Helsinki - Ethical principles for medical research involving human subjects. 2013;Available from: https://www.wma.net/policies-post/wma-declaration-of-helsinki-ethical-principles-for-medical-research-involving-human-subjects/ (Accessed on 12 July 2018).

4. Ministry of Health and Welfare. Ministerial ordinance on good clinical practice for drugs. Ordinance of the Ministry of Health and Welfare No. 28 of March 27, 1997, as last amended No. 161 of December 28, 2012. Provisional Translation (as of March 2013). 2013;Available from: https://www.pmda.go.jp/files/000152996.pdf (Accessed on 17 January 2020).

5. FDA (Food and Drug Administration). Guidance for industry - Modified risk tobacco product applications - Draft Guidance. 2012;Available from: https://www.fda.gov/media/83300/download (Accessed on 27 September 2019).

6. U.S. Department of Health and Human Services. How tobacco smoke causes disease: the biology and behavioral basis for smoking-attributable disease: a report of the Surgeon General. Atlanta, GA: US Department of Health and Human Services, Centers for Disease Control and Prevention, National Center for Chronic Disease Prevention and Health Promotion, Office on Smoking and Health. 2010.

7. U.S. Department of Health and Human Services. Smoking cessation: a report of the Surgeon General. Washington (DC): US Department of Health and Human Services; 2020.

8. Messner B, Bernhard D. Smoking and cardiovascular disease: mechanisms of endothelial dysfunction and early atherogenesis. Arteriosclerosis, thrombosis, and vascular biology. 2014;34(3):509-15. Epub 2014/02/21. doi: 10.1161/atvbaha.113.300156. PubMed PMID: 24554606.

9. McEvoy JW, Blaha MJ, DeFilippis AP, Lima JA, Bluemke DA, Hundley WG, et al. Cigarette smoking and cardiovascular events: role of inflammation and subclinical

atherosclerosis from the MultiEthnic Study of Atherosclerosis. Arteriosclerosis, thrombosis, and vascular biology. 2015;35(3):700-9. Epub 2015/01/13. doi: 10.1161/atvbaha.114.304562. PubMed PMID: 25573855; PubMed Central PMCID: PMC4404404.

10. McEvoy JW, Nasir K, DeFilippis AP, Lima JA, Bluemke DA, Hundley WG, et al. Relationship of cigarette smoking with inflammation and subclinical vascular disease: the Multi-Ethnic Study of Atherosclerosis. Arteriosclerosis, thrombosis, and vascular biology. 2015;35(4):1002-10. Epub 2015/03/07. doi: 10.1161/atvbaha.114.304960. PubMed PMID: 25745060; PubMed Central PMCID: Pmc4484586.

11. Morris PB, Ference BA, Jahangir E, Feldman DN, Ryan JJ, Bahrami H, et al. Cardiovascular effects of exposure to cigarette smoke and electronic cigarettes: clinical perspectives from the Prevention of Cardiovascular Disease Section Leadership Council and Early Career Councils of the American College of Cardiology. J Am Coll Cardiol. 2015;66(12):1378-91. Epub 2015/09/19. doi: 10.1016/j.jacc.2015.07.037. PubMed PMID: 26383726.

12. Hackshaw A, Morris JK, Boniface S, Tang JL, Milenkovic D. Low cigarette consumption and risk of coronary heart disease and stroke: meta-analysis of 141 cohort studies in 55 study reports. Bmj. 2018;360:j5855. Epub 2018/01/26. doi: 10.1136/bmj.j5855. PubMed PMID: 29367388; PubMed Central PMCID: PMC5781309.

13. Ambrose JA, Barua RS. The pathophysiology of cigarette smoking and cardiovascular disease: an update. J Am Coll Cardiol. 2004;43(10):1731-7. doi: 10.1016/j.jacc.2003.12.047. PubMed PMID: 15145091.

14. Rigotti NA, Clair C. Managing tobacco use: the neglected cardiovascular disease risk factor. Eur Heart J. 2013;34(42):3259-67. Epub 2013/09/10. doi: 10.1093/eurheartj/eht352. PubMed PMID: 24014389.

15. Weintraub WS, Luscher TF, Pocock S. The perils of surrogate endpoints. Eur Heart J. 2015;36(33):2212-8. Epub 2015/05/16. doi: 10.1093/eurheartj/ehv164. PubMed PMID: 25975658; PubMed Central PMCID: PMC4554958.

16. Ras RT, Streppel MT, Draijer R, Zock PL. Flow-mediated dilation and cardiovascular risk prediction: a systematic review with meta-analysis. International journal of cardiology. 2013;168(1):344-51. Epub 2012/10/09. doi: 10.1016/j.ijcard.2012.09.047. PubMed PMID: 23041097.

17. Inaba Y, Chen JA, Bergmann SR. Prediction of future cardiovascular outcomes by flow-mediated vasodilatation of brachial artery: a meta-analysis. The international journal of

cardiovascular imaging. 2010;26(6):631-40. Epub 2010/03/27. doi: 10.1007/s10554-010-9616-1. PubMed PMID: 20339920.

18. Nurnberger J, Keflioglu-Scheiber A, Opazo Saez AM, Wenzel RR, Philipp T, Schafers RF. Augmentation index is associated with cardiovascular risk. Journal of hypertension. 2002;20(12):2407-14. Epub 2002/12/11. doi: 10.1097/00004872-200212000-00020. PubMed PMID: 12473865.

19. Willum-Hansen T, Staessen JA, Torp-Pedersen C, Rasmussen S, Thijs L, Ibsen H, et al. Prognostic value of aortic pulse wave velocity as index of arterial stiffness in the general population. Circulation. 2006;113(5):664-70. Epub 2006/02/08. doi: 10.1161/circulationaha.105.579342. PubMed PMID: 16461839.

20. Laurent S, Cockcroft J, Van Bortel L, Boutouyrie P, Giannattasio C, Hayoz D, et al. Expert consensus document on arterial stiffness: methodological issues and clinical applications. Eur Heart J. 2006;27(21):2588-605. Epub 2006/09/27. doi: 10.1093/eurheartj/ehl254. PubMed PMID: 17000623.

21. U.S. Department of Health and Human Services. The health consequences of smoking - A report of the Surgeon General. Atlanta, GA: US Department of Health and Human Services, Centers for Disease Control and Prevention, National Center for Chronic Disease Prevention and Health Promotion, Office on Smoking and Health. 2004.

22. Danesh J, Collins R, Appleby P, Peto R. Association of fibrinogen, C-reactive protein, albumin, or leukocyte count with coronary heart disease: meta-analyses of prospective studies. JAMA. 1998;279(18):1477-82. Epub 1998/05/26. PubMed PMID: 9600484.

23. Koo HK, Kang HK, Song P, Park HK, Lee SS, Jung H. Systemic white blood cell count as a biomarker associated with severity of chronic obstructive lung disease. Tuberc Respir Dis (Seoul). 2017;80(3):304-10. Epub 2017/07/28. doi: 10.4046/trd.2017.80.3.304. PubMed PMID: 28747965; PubMed Central PMCID: PMC5526959.

24. Gan WQ, Man SF, Senthilselvan A, Sin DD. Association between chronic obstructive pulmonary disease and systemic inflammation: a systematic review and a meta-analysis. Thorax. 2004;59(7):574-80. Epub 2004/06/30. doi: 10.1136/thx.2003.019588. PubMed PMID: 15223864; PubMed Central PMCID: PMC1747070.

25. Kinnula VL, Ilumets H, Myllärniemi M, Sovijärvi A, Rytilä P. 8-Isoprostane as a marker of oxidative stress in nonsymptomatic cigarette smokers and COPD. Eur Respir J. 2007;29(1):51-5. Epub 2006/10/20. doi: 10.1183/09031936.00023606. PubMed PMID: 17050565.

26. Wong JYY, Bassig BA, Loftfield E, Hu W, Freedman ND, Ji BT, et al. White blood cell count and risk of incident lung cancer in the UK biobank. JNCI Cancer Spectr. 2020;4(2):pkz102. Epub 2020/12/15. doi: 10.1093/jncics/pkz102. PubMed PMID: 33313477; PubMed Central PMCID: PMC7083262.

27. Yuan JM, Carmella SG, Wang R, Tan YT, Adams-Haduch J, Gao YT, et al. Relationship of the oxidative damage biomarker 8-epi-prostaglandin F2α to risk of lung cancer development in the Shanghai Cohort Study. Carcinogenesis. 2018;39(7):948-54. Epub 2018/05/05. doi: 10.1093/carcin/bgy060. PubMed PMID: 29726912; PubMed Central PMCID: PMC7190890.

28. Nour Eldin EEM, Nour Eldein MM, El-Readi MZ, Mirza AA, Fatani SH, Al-Amodi HS, et al. Evaluation of the diagnostic and predicative values of 8-iso-prostaglandin F2α as a biomarker of breast cancer. Oncol Res Treat. 2020;43(10):506-17. Epub 2020/07/30. doi: 10.1159/000509671. PubMed PMID: 32721979.

29. Wong ET, Kogel U, Veljkovic E, Martin F, Xiang Y, Boue S, et al. Evaluation of the Tobacco Heating System 2.2. Part 4: 90-day OECD 413 rat inhalation study with systems toxicology endpoints demonstrates reduced exposure effects compared with cigarette smoke. Regul Toxicol Pharmacol. 2016;Suppl 2. Available from: http://www.sciencedirect.com/science/article/pii/S027323001630304X (Accessed on 03 May 2017):S59-81. Epub 2016/10/30. doi: 10.1016/j.yrtph.2016.10.015. PubMed PMID: 27793746.

30. Oviedo A, Lebrun S, Kogel U, Ho J, Tan WT, Titz B, et al. Evaluation of the Tobacco Heating System 2.2. Part 6: 90-day OECD 413 rat inhalation study with systems toxicology endpoints demonstrates reduced exposure effects of a mentholated version compared with mentholated and non-mentholated cigarette smoke. Regul Toxicol Pharmacol. 2016;Suppl 2. Available from: http://www.sciencedirect.com/science/article/pii/S0273230016303324 (Accessed on 03 May 2017):S93-122. Epub 2016/11/08. doi: 10.1016/j.yrtph.2016.11.004. PubMed PMID: 27818348.

31. Phillips B, Veljkovic E, Boue S, Schlage WK, Vuillaume G, Martin F, et al. An 8-month systems toxicology inhalation/cessation study in Apoe-/- mice to investigate cardiovascular and respiratory exposure effects of a candidate modified risk tobacco product, THS 2.2, compared with conventional cigarettes. Toxicological sciences : an official journal of the Society of Toxicology. 2016;149(2):411-32. Epub 2015/11/27. doi: 10.1093/toxsci/kfv243. PubMed PMID: 26609137; PubMed Central PMCID: Pmc4725610.

32. Philip Morris Products S.A. Nicotine pharmacokinetic profile and safety of the Tobacco Heating System 2.2 (THS 2.2)[ZRHR-PK-01-EU].In: ClinicalTrials.gov [Internet]. Bethesda

(MD): National Library of Medicine (US). 2013-2014 [cited 2015 Jun 16]. Available from: http://clinicaltrials.gov/show/NCT01967732 NLM Identifier: NCT01967732.

33. Philip Morris Products S.A. A single-center, open-label, randomized, controlled, crossover study to explore the nicotine pharmacokinetic profile and safety of Tobacco Heating System (THS) 2.1 compared to conventional cigarettes following single and ad libitum use in smoking, but otherwise healthy subjects [ZRHX-PK-02].In: ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US). 2012 [cited 2015 Jun 15]. Available from: http://clinicaltrials.gov/show/NCT01780688 NLM Identifier: NCT01780688.

34. Philip Morris Products S.A. A single-center, randomized, controlled, crossover study to investigate the nicotine pharmacokinetic profile and safety of THS 2.2 Menthol following single use in smokers compared to menthol conventional cigarettes and nicotine gum [ZRHM-PK-05-JP].In: ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US). 2013-2014 [cited 2016 Jan 03]. Available from: http://clinicaltrials.gov/show/NCT01967706 NLM Identifier: NCT01967706.

35. Philip Morris Products S.A. A single-center, randomized, controlled, crossover study to investigate the nicotine pharmacokinetic profile and safety of THS 2.2 Menthol following single use in smokers compared to menthol conventional cigarettes and nicotine nasal spray [ZRHM-PK-06-US].In: ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US). 2013-2014 [cited 2015 Jun 16]. Available from: http://clinicaltrials.gov/show/NCT01967719 NLM Identifier: NCT01967719.

36. Philip Morris Products S.A. A randomized, controlled, open-label, 3-arm parallel group, single center study to demonstrate reductions in exposure to selected smoke constituents in smoking, healthy subjects switching to the THS 2.2 or smoking abstinence, compared to continuing to use conventional cigarettes, for 5 days in confinement (ZRHR-REXC-03-EU).In: ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US). 2013-2014 [cited 2015 Jun 16]. Available from: http://clinicaltrials.gov/show/NCT01959932 NLM Identifier: NCT01959932.

37. Philip Morris Products S.A. A controlled, 3-arm parallel group study to demonstrate reductions in exposure to smoke constituents in smoking subjects switching to THS 2.2 or to smoking abstinence, compared to smoking conventional cigarettes for 5 days in confinement [ZRHR-REXC-04-JP].In: ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US). 2013-2014 [cited 2015 Jun 16]. Available from: http://clinicaltrials.gov/show/NCT01970982 NLM Identifier: NCT01970982.

38. Philip Morris Products S.A. Reduced exposure study using the Tobacco Heating System 2.2 (THS 2.2) Menthol for 91 days in confinement and ambulatory [ZRHM-REXA-08-US].In:

ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US). 2013-2015 [cited 2015 Jun 16]. Available from: http://clinicaltrials.gov/show/NCT01989156 NLM Identifier: NCT01989156.

39. Philip Morris Products S.A. A randomized, controlled, multi-center study to demonstrate reductions in exposure to selected smoke constituents in smokers switching to THS 2.2 Menthol or smoking abstinence compared to smoking menthol conventional cigarettes, for 90 days [ZRHM-REXA-07-JP].In: ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US). 2013-2014 [cited 2015 Jun 16]. Available from: http://clinicaltrials.gov/show/NCT01970995 NLM Identifier: NCT01970995.

40. Philip Morris Products S.A. Evaluation of biological and functional changes in healthy Smokers after switching to THS 2.2 for 26 weeks [ZRHR-ERS-09-US].In: ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US). 2015- [cited 2015 Aug 26]. Available from: https://clinicaltrials.gov/ct2/show/NCT02396381 NLM Identifier: NCT02396381.

41. Philip Morris S.A. A 26-week extension of the ZRHR-ERS-09-US study evaluating biological and functional changes in healthy smokers after switching to THS 2.2 [ZRHR-ERS-09-EXT-US].In: ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US). 2015- [cited 2016 Jun 01]. Available from: http://clinicaltrials.gov/show/NCT02649556 NLM Identifier: NCT02649556.

42. Lüdicke F, Ansari SM, Lama N, Blanc N, Bosilkovska M, Donelli A, et al. 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. Cancer Epidemiology, Biomarkers & Prevention. 2019;28(11):1934-43. doi: 10.1158/1055-9965.EPI-18-0915.

43. Philip Morris Products S.A. An observational cohort study in Japan to assess the patterns of product use and changes in health status associated with the use of HeatSticks with the THS 2.2 Tobacco Heating System [P1-PMC-01-JP].In: ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US). 2016- [cited 2017 Jan 16]. Available from: https://clinicaltrials.gov/ct2/show/NCT03020667 NLM Identifier: NCT03020667.

44. Philip Morris Products S.A. *Unpublished on file data:* Summary of product information (SPI) - Tobacco Heating System (THS). Version 5.0. 2019.

45. Raw M, Anderson P, Batra A, Dubois G, Harrington P, Hirsch A, et al. WHO Europe evidence based recommendations on the treatment of tobacco dependence. Tob Control. 2002;11(1):44-6. doi: 10.1136/tc.11.1.44. PubMed PMID: 11891367.

46. Japanese Circulation Society Joint Working Group. Guidelines for smoking cessation (JCS 2010) - Digest version. Availble from: https://www.jstage.jst.go.jp/article/circj/76/4/76_CJ-88-0021/_pdf (Accessed on 14 July 2017=. Circ J. 2012;76(4):1024-43. Epub 2012/02/14. PubMed PMID: 22327033.

47. Ministry of Health, Labour and Welfare. [Smoking cessation support manual (2nd edition)] Japanese. 2013;Available from: http://www.mhlw.go.jp/topics/tobacco/kin-en-sien/manual2/dl/manual2.pdf (Accessed on 14 July 2017).

48. Van Bortel LM, Balkestein EJ, van der Heijden-Spek JJ, Vanmolkot FH, Staessen JA, Kragten JA, et al. Non-invasive assessment of local arterial pulse pressure: comparison of applanation tonometry and echo-tracking. Journal of hypertension. 2001;19(6):1037-44. doi: 10.1097/00004872-200106000-00007.

49. Ludicke F, Magnette J, Baker G, Weitkunat R. A Japanese cross-sectional multicentre study of biomarkers associated with cardiovascular disease in smokers and non-smokers. Biomarkers. 2015;20(6-7):411-21. Epub 2015/12/01. doi: 10.3109/1354750x.2015.1096303. PubMed PMID: 26616146; PubMed Central PMCID: Pmc4720051.

50. Roethig HJ, Feng S, Liang Q, Liu J, Rees WA, Zedler BK. A 12-month, randomized, controlled study to evaluate exposure and cardiovascular risk factors in adult smokers switching from conventional cigarettes to a second-generation Electrically Heated Cigarette Smoking System. J Clin Pharmacol. 2008;48(5):580-91. Epub 2008/03/06. doi: 10.1177/0091270008315316. PubMed PMID: 18319361.

51. Lee PN, Forey BA, Fry JS, Thornton AJ, Coombs KJ. The effect of quitting smoking on white blood cell count - A review based on within-subject changes [Internet]. 2014;Available from: http://www.pnlee.co.uk/documents/refs/lee2014D.pdf (Accessed on 15 May 2014).

52. Elesber AA, Best PJ, Lennon RJ, Mathew V, Rihal CS, Lerman LO, et al. Plasma 8-iso-prostaglandin F2alpha, a marker of oxidative stress, is increased in patients with acute myocardial infarction. Free radical research. 2006;40(4):385-91. Epub 2006/03/07. doi: 10.1080/10715760500539154. PubMed PMID: 16517503.

53. Haswell LE, Papadopoulou E, Newland N, Shepperd CJ, Lowe FJ. A cross-sectional analysis of candidate biomarkers of biological effect in smokers, never-smokers and ex-smokers. Biomarkers. 2014:1-12. Epub 2014/05/24. doi: 10.3109/1354750x.2014.912354. PubMed PMID: 24854418.

54. Pilz H, Oguogho A, Chehne F, Lupattelli G, Palumbo B, Sinzinger H. Quitting cigarette smoking results in a fast improvement of in vivo oxidation injury (determined via plasma,

serum and urinary isoprostane). Thrombosis research. 2000;99(3):209-21. Epub 2000/08/16. doi: 10.1016/s0049-3848(00)00249-8. PubMed PMID: 10944241.

55. Oguogho A, Lupattelli G, Palumbo B, Sinzinger H. Isoprostanes quickly normalize after quitting cigarette smoking in healthy adults. Vasa. 2000;29(2):103-5. Epub 2000/07/20. doi: 10.1024/0301-1526.29.2.103. PubMed PMID: 10901086.

56. Hakim IA, Harris R, Garland L, Cordova CA, Mikhael DM, Sherry Chow HH. Gender difference in systemic oxidative stress and antioxidant capacity in current and former heavy smokers. Cancer Epidemiol Biomarkers Prev. 2012;21(12):2193-200. Epub 2012/10/04. doi: 10.1158/1055-9965.epi-12-0820. PubMed PMID: 23033455.

57. U.S. Department of Health and Human Services. The health consequences of smoking - 50 years of progress: a report of the Surgeon General. 2014.

58. Higuchi T, Omata F, Tsuchihashi K, Higashioka K, Koyamada R, Okada S. Current cigarette smoking is a reversible cause of elevated white blood cell count: Cross-sectional and longitudinal studies. Preventive medicine reports. 2016;4:417-22. doi: 10.1016/j.pmedr.2016.08.009.

59. McElroy JP, Carmella SG, Heskin AK, Tang MK, Murphy SE, Reisinger SA, et al. Effects of cessation of cigarette smoking on eicosanoid biomarkers of inflammation and oxidative damage. PloS one. 2019;14(6):e0218386. doi: 10.1371/journal.pone.0218386.

60. Yuan JM, Gao YT, Murphy SE, Carmella SG, Wang R, Zhong Y, et al. Urinary levels of cigarette smoke constituent metabolites are prospectively associated with lung cancer development in smokers. Cancer research. 2011;71(21):6749-57. Epub 2011/10/27. doi: 10.1158/0008-5472.can-11-0209. PubMed PMID: 22028322; PubMed Central PMCID: PMC3392910.

61. Peck MJ, Sanders EB, Scherer G, Ludicke F, Weitkunat R. Review of biomarkers to assess the effects of switching from cigarettes to modified risk tobacco products. Biomarkers. 2018:1-32. Epub 2018/01/04. doi: 10.1080/1354750x.2017.1419284. PubMed PMID: 29297706.

62. Sakaguchi C, Nagata Y, Kikuchi A, Takeshige Y, Minami N. Differences in levels of biomarkers of potential harm among users of a heat-not-burn tobacco product, cigarette smokers, and never-smokers in Japan: a post-marketing observational study. Nicotine Tob Res. 2021;8(7):1143-52. Epub 2021/01/28. doi: 10.1093/ntr/ntab014. PubMed PMID: 33502518.

63. Chang CM, Cheng YC, Cho TM, Mishina EV, Del Valle-Pinero AY, van Bemmel DM, et al. Biomarkers of potential harm: summary of an FDA-sponsored public workshop. Nicotine

Tob Res. 2019;21(1):3-13. Epub 2017/12/19. doi: 10.1093/ntr/ntx273. PubMed PMID: 29253243.

64. Akiyama Y, Sherwood N. Systematic review of biomarker findings from clinical studies of electronic cigarettes and heated tobacco products. Toxicology reports. 2021;8:282-94. doi: 10.1016/j.toxrep.2021.01.014.

65. Jatoi NA, Jerrard-Dunne P, Feely J, Mahmud A. Impact of smoking and smoking cessation on arterial stiffness and aortic wave reflection in hypertension. Hypertension. 2007;49(5):981-5. Epub 2007/03/21. doi: 10.1161/hypertensionaha.107.087338. PubMed PMID: 17372029.

66. Li H, Srinivasan SR, Berenson GS. Comparison of the measures of pulsatile arterial function between asymptomatic younger adult smokers and former smokers: the Bogalusa Heart Study. Am J Hypertens. 2006;19(9):897-901. Epub 2006/09/01. doi: 10.1016/j.amjhyper.2006.02.004. PubMed PMID: 16942930.

67. Janner JH, Godtfredsen NS, Ladelund S, Vestbo J, Prescott E. Aortic augmentation index: reference values in a large unselected population by means of the SphygmoCor device. Am J Hypertens. 2010;23(2):180-5. Epub 2009/12/05. doi: 10.1038/ajh.2009.234. PubMed PMID: 19959999.

68. FDA (Food and Drug Administration). Guidance for industry - Patient-reported outcome measures: use in medical product development to support labeling claims. 2009.

69. Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, et al. Standardisation of spirometry. Eur Respir J. 2005;26(2):319-38. Epub 2005/08/02. doi: 10.1183/09031936.05.00034805. PubMed PMID: 16055882.

70. Quanjer PH, Stanojevic S, Cole TJ, Baur X, Hall GL, Culver BH, et al. Multi-ethnic reference values for spirometry for the 3-95-yr age range: the global lung function 2012 equations. Eur Respir J. 2012;40(6):1324-43. Epub 2012/06/30. doi: 10.1183/09031936.00080312. PubMed PMID: 22743675; PubMed Central PMCID: PMC3786581.

71. Alguren B, Weitkunat R. Perception and prevalence of behavioral risk factors: the lifestyle risk scale (LRS). Open journal of preventive medicine. 2011;1(3):143-53.

72. Hanibuchi T, Nakaya T, Murata C. Socio-economic status and self-rated health in East Asia: a comparison of China, Japan, South Korea and Taiwan. Eur J Public Health. 2012;22(1):47-52. doi: 10.1093/eurpub/ckq174. PubMed PMID: 21113030.

73. European Social Survey. European Social Survey; Education Upgrade ESS1-ESS4 Documentation Report, The ESS Data Archive, Edition 1.3.Available from: https://www.europeansocialsurvey.org/data/themes.html?t=sociodemo (Accessed on 16 April 2020).

74. Festy P, Gaymu J, Thevenin M. Assessing the houshold 's financial situation, alone with the interviewer or in the partner's presence. [Original in French: Translated from French by Catriona Dutreuilh]. Population. 2014:81-101. doi: 10.3917/popu.1401.0085.

75. Dehghan M, Mente A, Teo KK, Gao P, Sleight P, Dagenais G, et al. Relationship between healthy diet and risk of cardiovascular disease among patients on drug therapies for secondary prevention: a prospective cohort study of 31 546 high-risk individuals from 40 countries. Circulation. 2012;126(23):2705-12. Epub 2012/12/06. doi: 10.1161/circulationaha.112.103234. PubMed PMID: 23212996.

76. Chrea C, Acquadro C, Afolalu EF, Spies E, Salzberger T, Abetz-Webb L, et al. Developing fit-for-purpose self-report instruments for assessing consumer responses to tobacco and nicotine products: the ABOUT Toolbox initiative. F1000Res. 2018;7:1878. Epub 2019/03/25. doi: 10.12688/f1000research.16810.1. PubMed PMID: 30906527; PubMed Central PMCID: PMC6415329.

77. Cano S, Chrea C, Salzberger T, Alfieri T, Emilien G, Mainy N, et al. Development and validation of a new instrument to measure perceived risks associated with the use of tobacco and nicotine-containing products. Health and Quality of Life Outcomes. 2018;16(1):192. doi: 10.1186/s12955-018-0997-5.

78. Salzberger T, Cano S, Abeth-Webb L, Afolalu E, Chrea C, Weitkunat R, et al. Addressing traceability of self-reported dependence measurement through the use of crosswalks. Measurement. 2021:109593. doi: https://doi.org/10.1016/j.measurement.2021.109593.

79. Afolalu EF, Spies E, Bacso A, Clerc E, Abetz-Webb L, Gallot S, et al. Impact of tobacco and/or nicotine products on health and functioning: a scoping review and findings from the preparatory phase of the development of a new self-report measure. Harm Reduction Journal. 2021;18(1):79. doi: 10.1186/s12954-021-00526-z.

80. Ware JA, Kosinski M, Bjorner J, Turner-Bowker D, Gandek B, Maruish M. User's Manual for the SF-36v2® Health Survey. 2nd ed. Incorporated Q, editor. Lincoln2017.

81. ICH E6(R2). Guideline for good clinical practice. 2016;Available from: https://www.ema.europa.eu/en/documents/scientific-guideline/ich-e-6-r2-guideline-good-clinical-practice-step-5_en.pdf (Accessed on 17 January 2010).

82. Lee GB, Shim JS, Kim HC. Dose-response association between smoking cessation and arterial stiffness: the Cardiovascular and Metabolic Diseases Etiology Research Center (CMERC) cohort. Korean circulation journal. 2020;50(4):361-9. Epub 2020/01/22. doi: 10.4070/kcj.2019.0270. PubMed PMID: 31960641; PubMed Central PMCID: PMC7067604.

83. Schmidt KMT, Hansen KM, Johnson AL, Gepner AD, Korcarz CE, Fiore MC, et al. Longitudinal effects of cigarette smoking and smoking cessation on aortic wave reflections, pulse wave velocity, and carotid artery distensibility. Journal of the American Heart Association. 2019;8(24):e013939. Epub 2019/12/05. doi: 10.1161/jaha.119.013939. PubMed PMID: 31795823; PubMed Central PMCID: PMC6951052.

84. Minami J, Ishimitsu T, Ohrui M, Matsuoka H. Association of smoking with aortic wave reflection and central systolic pressure and metabolic syndrome in normotensive Japanese men. Am J Hypertens. 2009;22(6):617-23. Epub 2009/03/28. doi: 10.1038/ajh.2009.62. PubMed PMID: 19325535.

85. Rostron BL, Corey CG, Chang JT, van Bemmel DM, Miller ME, Chang CM. Associations of cigarettes smoked per day with biomarkers of exposure among US adult cigarette smokers in the population assessment of tobacco and health (PATH) study wave 1 (2013-2014). Cancer Epidemiol Biomarkers Prev. 2019. Epub 2019/06/27. doi: 10.1158/1055-9965.Epi-19-0013. PubMed PMID: 31239264.

86. ICH E3. Structure and content of clinical study reports - Guideline. 1995;Available from: http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Efficacy/E3/E3_Guideline.pdf (Accessed on 17 July 2014).

15 APPENDICES

APPENDIX A SCHEDULE OF EVENTS

	Visit 1	Safety Follow-up
Study Day	**Day 1**	**Day 2**
Prior to Enrollment		
Informed consent [a]	●	
Vital signs	●	
Information on the risks of smoking and smoking/THS cessation advice	●	
Demographics [b]	●	
Medical history/concomitant diseases	●	
Prior/concomitant medication	●	●[d]
Questions on tobacco/nicotine-containing products use history	●	
Body height, weight and BMI	●	
CO breath test	●	
Alcohol breath test	●	
U: Drug screen [c]	●	
U: Cotinine test (cut-off ≥ 200 ng/mL or < 100 ng/mL) [c]	●	
U: Pregnancy test (only female subjects) [c]	●	
Physical examination	●	
ECG	●	
Spirometry pre- and post-BD	●	
Inclusion/exclusion criteria	●	
Enrollment	●	
After Enrollment		
WHR	●	

	Visit 1	Safety Follow-up
Study Day	**Day 1**	**Day 2**
B: BoPH, BoExp and HbA1c	●	
U: Creatinine, BoPH and BoExp to HPHCs/nicotine [c]	●	
Lifestyle Questionnaire	●	
Qualitative food group frequency questionnaire	●	
Questions on socioeconomic indicators	●	
ABOUT–Perceived risk	●	
ABOUT–Dependence	●	
ABOUT–Health and Functioning	●	
SF-36	●	
Arterial Stiffness (AIx and PWV) assessment	●	
B: Biobanking (if optional ICF(s) signed)	●	
AE/SAE recording	●	●
Discharge	●	

a. The informed consent process can be started as soon as the subject is invited to participate in the study, but the ICF must be signed before the first assessment at Visit 1.
b. Sex, age and race.
c. The same spot urine collection will be used for pregnancy (all females), drug screen and cotinine tests, creatinine and for BoExp to HPHCs/nicotine. Sampling for BoExp should be performed as soon as possible, and samples frozen immediately. If enough urine could be collected, the same spot urine will be used for BoPH. Otherwise another spot urine collection will be performed later during the visit. Urine samples should be processed according to laboratory manual/SHM.
d. Medication administered as treatment for an AE.

Abbreviations:

AE = Adverse event; AIx: Augmentation Index; B: Blood sample required; BMI = Body mass index; BoPH= Biomarkers of Potential Harm; 2CyEMA=2-Cyanoethyl Mercapturic Acid N-Acetyl-S-(2-cyanoethyl)-L-cysteine (2CyEMA); CO = Carbon monoxide; ECG = Electrocardiogram; PWV= Pulse Wave Velocity; SAE = Serious adverse event; SF-36=36-Item Short Form Health Survey; total NNAL= total 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol; U = Urine sample required; VAS = Visual analog scale; WHR – Waist to Hip ratio.

APPENDIX B MEDICATIONS WITH IMPACT ON PRIMARY OBJECTIVE EVALUATION

Drug		WBC	8-epi-$PGF_{2\alpha}$	Last Dose Applied Prior to Lab Value Tested (5 x Half-Life)
Non-steroidal anti-inflammatory drugs (NSAID)	Ibuprofen, Diclofenac	●		≈ 1 day
	Celecoxib, Indometacin, Ketoprofen	●		≈ 2 days
	Meloxicam, Naproxen	●		≈ 4 days
	Piroxicam	●		≈ 10 days
Antivirals/antibiotics		●		
Antiplatelet agents	Acetylsalicylic acid		●	≈ 6 days
	Warfarin		●	≈ 9 days
	Apixaban, Rivaroxaban		●	≈ 3 days
	Phenprocoumon, Alpha-Tocopherol (Vitamin E)		●	≈ 35 days
Antidepressant drug	Bupropion	●		≈ 4 days
	Selective serotonin reuptake inhibitors (SSRIs)			
	Fluvoxamine	●		≈ 3 days
	Paroxetine	●		≈ 4 days
	Sertraline	●		≈ 5 days
	Citalopram, Escitalopram	●		≈7 days
	Fluoxetine	●		Initial: ≈ 15 days Chronic: ≈ 30 days
	Serotonin–norepinephrine reuptake inhibitors (SNRIs)			
	Venlafaxine	●		≈ 1 day
	Tricyclic antidepressants			
	Tricyclic clomipramine	●		≈ 5 days
	Non-specified antidepressant drug class			
	Nefazodone	●		≈ 5 days