PMI RESEARCH & DEVELOPMENT

Clinical Study Protocol

SA-SCR-01

Study Title:	A multi-center, multi-region smoking cessation study to understand the biological and functional changes related to smoking cessation in healthy smokers who are continuously abstinent from smoking for one year.
Short Title	A smoking cessation study to understand the biological and functional changes after one year of smoking cessation.
EUDRACT Number:	Not applicable
Product Name:	Not applicable
Study Number	SA-SCR-01
Sponsor:	Philip Morris Products S.A. Quai Jeanrenaud 5 2000 Neuchâtel Switzerland
Version Number:	Final Version 8.0
Revision Date:	18 May 2017
Authors:	[redacted], MD, MSc, Lead Clinical Scientist [redacted], PhD, PMP, Clinical Scientist [redacted] PhD, Biostatistician [redacted], MD, Medical Safety Officer

SUMMARY OF CHANGES

Clinical Study Protocol
SA-SCR-01

	Version	Date	Amendment
Non-substantial updates	Final Version 8.0	18 May 2017	Non-substantial updates
Current protocol	Final Version 7.0	21 April 2016	No. 3
Second amended protocol*	Final Version 6.0	7 March 2016	No. 2
First amended protocol	Final Version 5.0	30 July 2015	No. 1
First updated protocol	Final Version 4.0	19 February 2015	Non-substantial updates
Original protocol	Final Version 3.0	24 November 2014	

*It should be noted that the second amended protocol (Final Version 6.0) dated 7 March 2016 was only submitted in Poland. Shortly after its submission, since other changes were required, the submission process in the other countries was stopped. The current protocol (Final Version 7.0) includes both the substantial changes between the first amended protocol (Final Version 5.0 – Amendment No. 1) and the second amended protocol (Final Version 6.0 – Amendment No. 2) and between the second amended protocol (Final Version 6.0 – Amendment No. 2) and the third amended protocol (Final Version 7.0 – Amendment No. 3).

INTRODUCTION

The main purpose of this summary of changes is:

To summarize

- the non-substantial updates between the clinical study protocol SA-SCR-01 (Final Version 3.0) dated 24 November 2014 and its first updated version (Final Version 4.0) dated 19 February 2015.

- the substantial changes between the Final Version 4.0 study protocol and the first amended protocol (Final Version 5.0) dated 30 July 2015 which is to be referred to Amendment No. 1.
- the substantial changes between the Final Version 5.0 study protocol (Amendment No. 1) and the second amendedprotocol (Final Version 6.0) dated 7 March 2016 which is to be referred to Amendment No. 2.
- the substantial changes between the Final Version 6.0 study protocol (Amendment No. 2) and the current protocol (Final Version 7.0) dated 21 April 2016 which is to be referred to Amendment No. 3.
- The non-substantial updates between the Final Version 7.0 study protocol dated 21 April 2016 and its updated version (Final Version 8.0) dated 18 May 2017.

More precise details on the protocol sections changed are provided. For identification of the changes, the previous and the amended texts are provided. The new text has been highlighted in bold (e.g. **new text**) and deleted text has been crossed out (e.g. ~~deleted text~~).

Section		Changes
From Final 7.0 to Final 8.0		
Front page	**Authors**	***Amended text:*** [redacted], MD, ~~PhD, MFPM,~~ Medical Safety Officer. ***Old text:*** [redacted], MD, PhD, MFPM, Medical Safety Officer. ***Reason to change:*** [redacted] replaced [redacted] as Medical Safety Officer.
8.3	**Reporting of Serious Adverse Events**	***Amended text:*** Sponsor Contact: [redacted], MD, ~~PhD, MFPM,~~ Medical Safety Officer. E-mail 2: [redacted].pmi.com ***Old text:*** Sponsor Contact: [redacted], MD, PhD, MFPM, Medical Safety Officer. E-mail 2: [redacted].pmi.com ***Reason to change:*** [redacted] replaced [redacted] as Medical Safety Officer.
From Final 6.0 to Final 7.0		
The current version (Final Version 7.0) dated 21 April 2016 is to be referred to Amendment No. 3.		
From Final 5.0 to Final 6.0		
The second amended protocol (Final Version 6.0) dated 7 March 2016 is to be referred to Amendment No. 2.		
From Final 4.0 to Final 5.0		
The first amended protocol (Final Version 5.0) dated 30 July 2015 is to be referred to Amendment No. 1.		
From Final 3.0 to Final 4.0		
	General	The version number and the revision date were updated accordingly to the most current version and date.
Synopsis	**Objectives and endpoints – 4. To monitor the safety**	***New text:*** **Body weight.** ***Reason to change:*** To add the parameter "body weight" to the list. "Body weight" was already listed as a parameter but missed in the list of the safety parameters.
3	**Study objectives and endpoints -**	***New text:*** **Body weight.**

Section		Changes
	4. To monitor the safety	***Reason to change:*** To add the parameter "body weight" to the list which was already listed as a parameter but missed in the list of the safety parameters.
List of appendices		List of appendices was updated with one new appendix.
List of abbreviations and definitions of terms		List of abbreviations was updated with a change of one abbreviation.
Explanation of Terms	**Lost to follow-up (date)**	***Amended text:*** **~~The date of~~ When the PI(s) or designee(s) declare(s) a subject is lost to follow-up ~~corresponds~~, the lost to follow-up date will be recorded and will correspond to the date of the ~~last record made by or for~~end of study of the subject.** **If the site has lost track of the subject but the subject has reached the maximum number of study days (434 days), then the PI(s) or designee(s) will declare the subject lost to follow-up at this date.** ***Old text:*** The date of lost to follow-up corresponds to the date of the last record made by or for the subject. ***Reason to change:*** To state more precisely on the date of the end of study of a subject that is considered as lost to follow-up and which dates are to be recorded.
4.1	**Overall Study Design**	***New text:*** **Once approximately 950 subjects will reach V5, screening and enrolment will be stopped. Subjects already enrolled in the study at that point in time and that are between V2 and V5, will be kept in the study and allowed to progress over Visit 5. On the contrary, on-going subjects that have completed screening visit and are before visit 2 will be discontinued from the study and classified as screen failures.** ***Reason to change:*** To clarify the precise procedure for the enrolment and discontinuation of subjects once the predefined number of the study population has been reached.
4.1	**From Check-out of V2 to Actual Quit Date**	***Amended text:*** Subjects will be asked to record their AQD **and to communicate this date to the site in order to schedule following visits**.

Section		Changes
		Old text: Subjects will be asked to record their AQD. ***Reason to change:*** To make sure that the date of AQD will be recorded by the site as all of the following visits will be scheduled according to the AQD communicated.
5.1.2	**Exclusion Criterion No 19**	***Amended text:*** For women only: subject is pregnant (does have positive pregnancy test ~~at V1~~) or is breast feeding. ***Old text:*** For women only: subject is pregnant (does have positive pregnancy test at V1) or is breast feeding. ***Reason to change:*** To ensure that the pregnancy test will not be done at V1 only.
5.1.2	**Exclusion Criterion No 19**	***Amended text:*** Exclusion criterion No 19 will be re-checked for eligibility at Visit 2 (Baseline). ***Old text:*** Exclusion criterion No 19 will be checked for eligibility at Visit 1 (Screening) only. ***Reason to change:*** To ensure that female subjects to be included have not become pregnant in the period between V1 and V2 as this period could last up to 42 days.
5.1.2	**Exclusion Criteria**	***Amended text:*** * Concomitant Medication with potential impact on Clinical Risk Endpoints (section 6.4). **Subjects using salbutamol for post-bronchodilator spirometry testing at Screening will not be excluded from the study.** ***Old text:*** * Concomitant Medication with potential impact on Clinical Risk Endpoints (section 6.4) ***Reason to change:*** Generally, Salbutamol is listed in Table 1 and should not be used within the last 42 days prior to enrolment in the study. However, Salbutamol used for spirometry testing at V1 is a procedural part of the study assessments, not as a treatment medication in any other indications.
5.3	**Discontinuation of Subjects**	***New text:*** Nicotine-containing products, other than NRT. ***Old text:*** Nicotine-containing product, other than NRT. ***Reason to change:*** It was an error in writing.
5.4	**Lost to Follow-up**	***Amended text:*** ~~The end of the study date for a subject lost to follow-up will be defined as the date of last contact. A reasonable~~**Reasonable** number of attempts to contact

Section		Changes
		the subject **(**including written correspondence and phone calls**)** should be ~~made~~**done** and documented in the source documents by the site. ~~The date when the Investigator~~**The date of the last contact (e.g. last visit, last phone call) should also be recorded in the source document. When the PI(s)** or designee ~~declares~~**(s)declare(s)** a subject is lost to follow-up**, the lost to follow-up date** will be recorded ~~as~~**and will correspond to** the date of **the end of study of the subject.** **If the site has lost track of the subject but the subject has reached the maximum number of study days (434 days), then the PI(s) or designee(s) will declare the subject** lost to follow-up **at this date**. ***Old text:*** The end of the study date for a subject lost to follow-up will be defined as the date of last contact. A reasonable number of attempts to contact the subject including written correspondence and phone calls should be made and documented in the source documents by the site. The date when the Investigator or designee declares a subject is lost to follow-up will be recorded as the date of lost to follow-up. ***Reason to change:*** To state more precisely on the date of the end of study of a subject that is considered as lost to follow-up and which dates are to be recorded.
6.2	**Compliance**	***Amended text:*** Continuous smoking abstinence will be ~~verified by~~ self-~~reporting (of product use in a diary or a form~~ **reported** by the subject~~)~~ and **recorded in the CRF, and verified by** exhaled CO breath test during the visits. **Old text:** Continuous smoking abstinence will be verified by self-reporting (of product use in a diary or a form by the subject) and exhaled CO breath test during the visits. ***Reason to change:*** For convenience of the subjects, the recording of continuous smoking abstinence in a diary or in a separate form is dropped.
6.4	**Concomitant Medication**	***Amended text:*** In case drugs/short-and long-term vitamins listed in Table 1 are taken by the subject from the enrolment onwards, they will be recorded as concomitant medications **(except when salbutamol is used for post-bronchodilator spirometry testing),** but this will not be a reason to discontinue the subject from the study.

Section		Changes
		Old text: In case drugs/short-and long-term vitamins listed in Table 1 are taken by the subject from the enrolment onwards, they will be recorded as concomitant medications but this will not be a reason to discontinue the subject from the study. ***Reason to change:*** To make sure that using salbutamol is a procedural part during spirometry testing. Salbutamol is not to be recorded as a concomitant medication.
6.4	**Concomitant Medications, Table 1**	***New text:*** (~~i~~.e.**g**.,…) ***Old text:*** (i.e.,….) ***Reason to change:*** It was an error in writing.
7.4.2	**Questions on Smoking History/** **Habits and Intention to Quit Smoking**	***New text:*** This self-reported CC daily consumption at V1 **and V2** will be used to assess eligibility. ***Old text:*** This self-reported CC daily consumption at V1 will be used to assess eligibility. ***Reason to change:*** The period between V1 and V2 could last up to 42 days. To evaluate that the daily conventional cigarette consumption habit has not changed during this period between V1 and V2, and the data recorded are still consistent.
7.4.3	**Medical History and Prior and Concomitant Medications**	***Amended text:*** A concomitant disease is defined as any condition that started prior to ~~V1 and~~ and is still ongoing **at V1, or is detected** at V1. ***Old text:*** A concomitant disease is defined as any condition that started prior to V1 and is still ongoing at V1. ***Reason to change:*** To clarify that diseases detected during V1 will be also recorded as concomitant diseases.
7.4.7.1	**Lung Function Testing** **Pre- and post-bronchodilator spirometry testing**	***Amended text:*** Pre and post- bronchodilator spirometry ~~tests~~ **assessments** will be performed. Each assessment requires at least three valid spirometry tests. ***Old text:*** Pre and post- bronchodilator spirometry tests will be performed. ***Reason to change:*** To specify the number of tests required for the acceptability of each assessment. ***Amended text:*** In case~~the value of the test(s)~~ tests do not meet the **acceptability** criteria the subject will need to come back within a 5-day window to repeat the ~~test(s).~~

Section		Changes
		tests. **More details will be provided in the Pulmonary Function Testing (PFT) manual.** ***Old text:*** In case the value of the test(s) do not meet the criteria the subject will need to come back within a 5-day window to repeat the test(s). ***Reason to change:*** To explain the procedure in more details for a better understanding and aligning with the PFT manual. ***Amended text:*** All post-bronchodilator spirometry testing will be performed 15-30 minutes post administration of around 400 µg of salbutamol/albuterol (usually equivalent to ~~2~~ **4** puffs assuming ~~90~~ **100** µg/puff). ***Old text:*** All post-bronchodilator spirometry testing will be performed 15-30 minutes post administration of around 400 µg of salbutamol/albuterol (usually equivalent to 2 puffs assuming 90 µg/puff). ***Reason to change:*** To correct the miscalculation.
7.6.2	**Sample Handling, Storage, and Shipment**	***Amended text:*** Detailed procedures for collection and handling of samples are described in a separate ~~sample handling~~ **Investigator Laboratory** Manual (~~SHM~~**ILM**). The bioanalytical lab(s) will be listed in the ~~SHM~~**ILM**. ***Old text:*** Detailed procedures for collection and handling of samples are described in a separate sample handling manual (SHM). The bioanalytical lab(s) will be listed in the SHM. ***Reason to change:*** The name of the manual is updated to make clear that the manual will be located at the site of the Investigator. ***Amended text:*** All **primary and back-up samples for the assessments of clinical risk endpoints and BoExp** ~~other samples~~ (except bio-banking samples) **of discontinued subjects** will be destroyed after **all** ~~the clinical study~~ **bioanalytical** reports ~~(CSR)~~ ha~~s~~**ve** been finalized **or the database has been locked, whichever comes last.** ***Old text:*** All other samples (except bio-banking samples) will be destroyed after the clinical study report (CSR) has been finalized.

Section		Changes
		Reason to change: To define more precisely which samples to be destroyed are meant, and to make sure that the next possible time point will be applied once the samples are no longer needed and to shorten the time of storage that is not necessary.
7.6.3	**Blood Samples**	***Amended text:*** The maximal total volume of blood drawn for each subject will be around 3~~50~~**75** mL, which includes about ~~40~~ **65** mL for safety and repeated analysis,… ***Old text:*** The maximal total volume of blood drawn for each subject will be around 350 mL, which includes about 40 mL for safety and repeated analysis,… ***Reason to change:*** To correct the miscalculation.
7.6.5	**Bio-banking for Long Term Storage of Blood and Urine**	***Amended text:*** Serum/plasma: at each visit 2 tubes with **about** 5 mL of blood **each** will be **filled**~~drawn~~ and centrifuged… ***Old text:*** Serum/plasma: at each visit 2 tubes with 5 mL of blood will be drawn and centrifuged… ***Reason to change:*** To clarify that the amount of blood sample might slightly vary during blood drawing for every tube.
7.7.1	**Questionnaires**	***Amended text:*** The questionnaires should be done at the same time for each visit. ***Old text:*** The questionnaires should be done at the same time for each visit. ***Reason to change:*** For the reason of practicability and to avoid the complexity without huge added value, the same time for answering the questionnaires at every visit was removed.
8.3	**Reporting of Serious Adverse Events**	***Amended text:*** **E-mail 1:** [redacted]**@pmi.com** E-mail **2**: [redacted]@pmi.com ***Old text:*** E-mail: [redacted]@pmi. ***Reason to change:*** To ensure that any safety related issue will be received in any case, the first email address was added.
8.4.1	**Abnormal Results of Laboratory Tests**	***Amended text:*** A grade 2 **and higher** laboratory abnormal value at V1 must be recorded as concomitant disease.

Section		Changes
		Old text: A grade 2 laboratory abnormal value at V1 must be recorded as concomitant disease. ***Reason to change:*** To be consistent in wording with other text sections already mentioning this classification.
9.2	**Table 4, Time Schedule – Baseline Visit (V2)**	***Amended text:*** During the visit ~~(prior to enrollment)~~ – Questions about smoking history/habits – ~~Must be done after enrollment~~ prior to enrollment. ***Old text:*** During the visit (prior to enrollment) – Questions about smoking history/habits – Must be done after enrollment. ***Reason to change:*** To correct the contradictory statements about the time of the assessments of the questions about smoking history/habits.
9.5	**Smoking Abstinence Period (V4 to V17) – Table 6 and Table 7**	***Amended text:*** During the visit - ~~SC support~~ **SC support and self-reporting by the subject on continuous smoking abstinence.** ***Old text:*** During the visit – SC support ***Reason to change:*** To make sure the self-reporting by the subject on continuous smoking abstinence.
9.6	**Early Termination Procedures**	***Amended text:*** Lung function (spirometry pre- and post-bronchodilator only; **In case the tests do not meet the acceptability criteria the subject will not need to repeat these tests)** ***Old text:*** Lung function (spirometry pre- and post-bronchodilator only). ***Reason to change:*** To clarify that the assessments of the lung function do not need to be repeated as these assessments are recorded only for safety reasons.
Appendices	**Appendix 2- Medications with Impact on Clinical Risk Endpoints (with half-lives)**	***Amended text:*** **Selective serotonin reuptake inhibitors** (SSRIs); **Serotonin–norepinephrine reuptake inhibitors (SNRIs)** ~~(NSRIs);~~ **Tricyclic antidepressants; Non-specified antidepressant drug class.** ***Old text:*** SSRIs; NSRIs ***Reason to change:*** Classification is updated to be more precise in categorizing the drugs listed.
Appendices	**Appendix 4 – Summary of**	***Amended text:*** 11-dehydrothromboxane B2 (11-DTX-B2) - ~~Endothelial dysfunction~~ **Platelet activation**

Section		Changes
	Clinical Risk Endpoints	***Old text:*** 11-dehydrothromboxane B2 (11-DTX-B2) - Endothelial dysfunction ***Reason to change:*** To correct the wrong categorization of the intended measurement.
Appendices	**Appendix 6- Abnormal Laboratory Values**	***Amended text:*** Those parameters that are not listed **and** do not have grading categories in the CTCAE will be reviewed by the ~~Principal~~ Investigator **or designee,** and will only be reported as an AE if considered to be clinically relevant. ***Old text:*** Those parameters that are not listed do not have grading categories in the CTCAE will be reviewed by the Principal Investigator and only reported as an AE if considered to be clinically relevant. ***Reason to change:*** To correct the wrong wording as the designee will also review the laboratory values and also report AEs where applicable.

SYNOPSIS

Sponsor:

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

Name of Product:

Not applicable

Study Title:

A multi-center, multi-region smoking cessation study to understand the biological and functional changes related to smoking cessation in healthy smokers who are continuously abstinent from smoking for one year.

Study Number:

SA-SCR-01

Study Short Title:

A smoking cessation study to understand the biological and functional changes after one year of smoking cessation.

Objectives and Endpoints:

The objectives and endpoints of this study are:

1. To describe the clinical, biological and functional changes in smokers who are continuously abstinent from smoking.

   Clinical risk endpoints associated with cardiovascular disease at week 13 (V8), week 26 (V11) and week 52 (V17):

   - White blood cell count (WBC), platelet count, glycosylated hemoglobin (HbA1c), and carboxyhemoglobin (COHb) in blood.
   - High and low density lipoprotein cholesterol (HDL-C, and LDL-C), myeloperoxidase (MPO), soluble intercellular adhesion molecule-1 (sICAM-1), apolipoprotein A1 and B (Apo A1 and Apo B), and high sensitivity C-reactive protein (hs-CRP) in serum.
   - Fibrinogen, and homocysteine in plasma.
   - Albumin, 11-dehydrothromboxane B2 (11-DTX-B2) and 8-epi-prostaglandin-alpha (8-epi-$PGF_{2\alpha}$) in urine (expressed as concentration adjusted to creatinine).

   Clinical risk endpoints associated with respiratory diseases:

   - Spirometry (pre- and post-bronchodilator): Forced expiratory volume in 1 second (FEV_1), forced vital capacity (FVC), FEV_1/FVC, forced expiratory flow (FEF 25-75), at week 13 (V8), week 26 (V11), and week 52 (V17).
   - Lung volume: vital capacity (VC), total lung capacity (TLC), functional residual capacity (FRC), inspiratory capacity (IC), at selected sites specialized for lung function testing, at week 13 (V8), week 26 (V11), and week 52 (V17).
   - Cough symptoms (intensity and frequency), amount of sputum production and bothersomeness of cough symptom from the cough questionnaire at week 13 (V8), week 26 (V11) and week 52 (V17).

   Clinical risk endpoint associated with xenobiotic metabolism at week 13 (V8), week 26 (V11), and week 52 (V17):

   - Cytochrome P450 2A6 (CYP2A6) activity: molar metabolic ratio of *trans*-3-hydroxycotinine/cotinine in plasma.

Clinical risk endpoint associated with genotoxicity at week 13 (V8), week 26 (V11), and week 52 (V17):

- Total 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (Total NNAL) in urine (expressed as concentration adjusted to creatinine).

2. To describe the changes in biomarkers of exposure (BoExp) to harmful and potentially harmful constituents (HPHCs) in smokers who are continuously abstinent from smoking.

   BoExp to HPHCs at week 13 (V8), week 26 (V11), and week 52 (V17):

   - BoExp to carbon monoxide (CO): CO in exhaled breath (expressed as ppm).*
   - BoExp to nicotine: cotinine and nicotine in plasma and nicotine equivalents (Neq) in urine[1].*
   - BoExp to 1,3-butadiene : monohydroxybutenylmercapturic acid (MHBMA).*
   - BoExp to acrolein: 3-hydroxypropylmercapturic acid (3-HPMA).*
   - BoExp to acrylonitrile: 2-cyanoethylmercapturic acid (CEMA).*
   - BoExp to benzo(a)pyrene: 3-hydroxybenzo(a)pyrene (B[a]P).*
   - BoExp to pyrene: Total 1-hydroxypyrene (Total 1-OHP).*
   - BoExp to crotonaldehyde: 3-hydroxy-1-methylpropylmercapturic acid (3-HMPMA).*
   - BoExp to N-nitrosonornicotine: total N-nitrosonornicotine (Total NNN).*
   - BoExp to 4-aminobiphenyl: 4-aminobiphenyl (4-ABP).
   - BoExp to benzene: S-phenylmercapturic acid (S-PMA).
   - BoExp to 1-aminonaphthalene: 1-aminonaphthalene (1-NA).
   - BoExp to 2-aminonaphthalene: 2-aminonaphthalene (2-NA).
   - BoExp to o-toluidine: o-toluidine (o-tol).
   - BoExp to ethylene oxide: 2-hydroxyethylmercapturic acid (HEMA).

[1] Nicotine equivalents (Neq) are defined as molar sum of free nicotine, nicotine-glucuronide, free cotinine, cotinine-glucuronide, free *trans*-3'-hydroxycotinine, *trans*-3'- hydroxycotinine-glucuronide.

- BoExp to toluene: S-benzylmercapturic acid (S-BMA).

All BoExp measured in urine, will be expressed as concentrations adjusted to creatinine. Only BoExp marked with " * " will be assessed at week 26 (V11) and week 52 (V17).

3. To describe the rate of continuous smoking abstinence at each visit following the actual quit date of smoking cessation (defined as AQD).

4. To monitor the safety:

   - Adverse events (AEs) / serious adverse events (SAEs).
   - Body weight.
   - Vital signs.
   - Spirometry
   - Electrocardiogram (ECG).
   - Clinical chemistry, hematology and urine analysis safety panel.
   - Physical examination.
   - Concomitant medications.

Additional study assessments:

- Prochaska "Stage of change" questionnaire.
- Fagerström test for nicotine dependence (FTND, revised version).
- Socio-economic status (in the following countries: US, UK, Poland, Germany and Japan).
- Lifestyle assessments.

Study Hypothesis:

Not applicable

Evaluation Criterion:

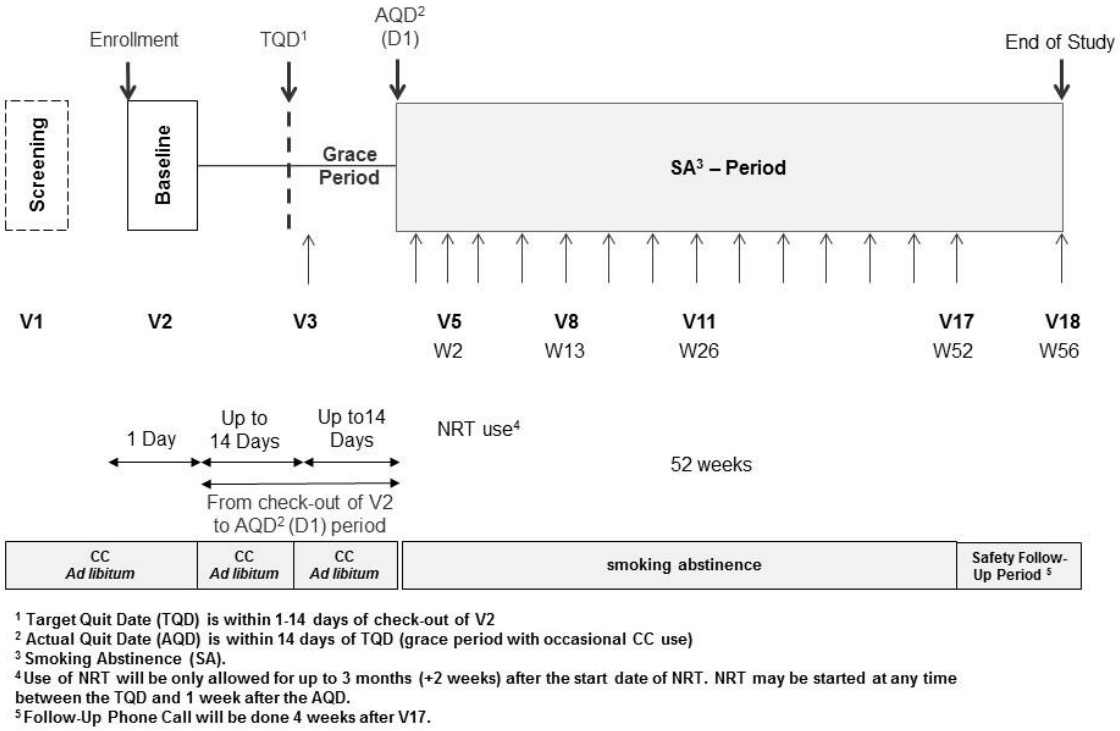
Not applicable

Study Design

This multi-region, multi-center, ambulatory study will be conducted in the US, Japan and Europe. Smokers who are motivated to quit smoking within the next 30 days at screening will be enrolled to reach approximately 950 subjects continuously abstinent from smoking from the actual quit date (AQD) onwards at week 2 (V5) in order to achieve at least 190 successful quitters to complete the study.

Smokers who are not continuously abstinent from smoking (*i.e.,* free from tobacco product use (*e.g*., CC, pipes, cigars, snus) or any nicotine containing product (including electronic cigarettes) other than nicotine replacement therapy (NRT)), as assessed by the four criteria described in section 6.2, from their AQD onwards will be discontinued from the study.

NRT use will be allowed as per label for up to 3 months (+2 weeks) after the start date of NRT to support the subject to remain abstinent from smoking. NRT may be started at any time between the TQD and 1 week after the AQD. The start day of NRT will be counted as Day 1 of NRT use. From that day on, the duration of NRT use must not exceed 3 months + 2 weeks. NRT will be reimbursed.



[1] Target Quit Date (TQD) is within 1-14 days of check-out of V2
[2] Actual Quit Date (AQD) is within 14 days of TQD (grace period with occasional CC use)
[3] Smoking Abstinence (SA).
[4] Use of NRT will be only allowed for up to 3 months (+2 weeks) after the start date of NRT. NRT may be started at any time between the TQD and 1 week after the AQD.
[5] Follow-Up Phone Call will be done 4 weeks after V17.

Abbreviations: CC = conventional cigarettes; NRT = nicotine replacement therapy; SA = smoking abstinence; V = visit; W = week.

Figure 1 Study Design

Screening Period

The Screening Period will take place 1 to 42 days prior to enrolment at V2 (Baseline). Eligibility of the subjects to participate in the study will be assessed during the Screening Visit (V1). Subjects who are still eligible at the end of V1 will be provided with urine containers and instructions for the 24-hour urine home collection. Eligible subjects will be contacted prior to V2 to confirm their eligibility and to take an appointment for V2.

Baseline Visit - V2 (from check-in to check-out from the site)

Twenty-four-hour urine collection will start in the morning of the day prior to V2 and end 24 hours later in the morning of V2. Enrolment of the subject will take place after the subject's check-in on site with the cooled urine container(s) filled with his/her 24-hour urine and re-check of inclusion criteria n°4 and n°6. All other procedures and data collection will be completed following the enrolment of the subject. All subjects will continue smoking their preferred brand of CC.

Before check-out, subjects will be asked to define their target quit date (TQD), the date from which the subject will stop smoking. The TQD must be within 14 days after V2.

From Check-out of V2 to Actual Quit Date (Day 1)

This period aims to identify subjects who are more motivated and more likely to quit as well as to remain continuously abstinent from smoking for the whole duration of the study. The period starts from check-out of V2 and ends with the AQD (Day 1) including the TQD, V3 and the Grace Period. This period might last up to 28 days for each subject.

The subject will be asked to come to the clinic for V3 within 24 to 48 hours after their defined TQD. The goal of this visit will be to ensure that the subject has actually quit smoking and to provide him/her with the necessary support. From V3 onwards, smoking cessation (SC) counseling and behavioral support will be provided to the subject according to the SC support plan. Additional SC support will be offered at any time as requested by the subject.

A Grace Period of a maximum of 14 days will be allowed after the TQD, during which occasional slips of smoking (defined as occasional use of nicotine and/or tobacco-containing products) will be accepted. From the AQD onwards, strict abstinence from any tobacco- or nicotine-containing product (including electronic cigarettes) other than NRT is required. Subjects will be asked to record their AQD and to communicate this date to the site in order to schedule following visits. The latest possible day for the AQD to occur is defined as the last day of the Grace Period (i.e., TQD + 14 days).

The Smoking Abstinence Period (from the AQD up to the check-out of V17 [week 52])

From the AQD, subjects will be asked to come on site for at week 1 (V4), at week 2 (V5) and then on a monthly basis at week 4 (V6), at week 9 (V7), at week 13 (V8), at week 17 (V9), at week 22 (V10), at week 26 (V11), at week 30 (V12), at week 35 (V13), at week 39 (V14), at week 43 (V15), at week 48 (V16), and at week 52 (V17). Visits will be scheduled based on the AQD. A time window of ± 8 days is allowed for the visits, with the exception of V4 (± 3 days) and V5 (± 3 days).

The V8, V11, and V17 will correspond to full assessment visits at site(s) where 24-hour urine and blood sampling will be collected for analysis of biomarkers of exposure (BoExp) and clinical risk endpoints. The collection of the 24-hour urine will start at home in the morning the day before the visit and will end 24 hours later in the morning of the day of the visit to the clinic.

The Safety Follow-Up Period and Phone Contact (28 days after the check-out of V17 [V18 (±3 days); week 56])

A subject who has completed V17, or a subject who has been discontinued from the study prematurely (early termination), will enter a 28-days Safety Follow-Up Period during which spontaneously reported new AEs/SAEs will be recorded, and the active follow-up of ongoing AEs/SAEs will be done by the site. All AEs will be followed-up until resolved, stabilized (*i.e.*, no worsening of the event), or a plausible explanation for the event has been found until the end of the Safety Follow-Up Period.

At the end of the Safety Follow-Up Period in Week 56 (V18 (±3 days)), the investigator will attempt to contact only the subject who has previously completed V17 by phone to check if all AEs/SAEs potentially occurring during the Safety Follow-Up period are fully reported and for self-reporting by the subject on continuous smoking abstinence. At the end of the Safety Follow-Up Period, all ongoing AEs will be documented as "ongoing" and will not be followed-up by the Investigator. At the discretion of the Investigator, the subject will be referred to his/her General Practitioner for follow up on ongoing AEs.

If the investigator can reach the subject who has previously completed V17 by phone at the end of the Safety Follow-Up Period in Week 56, the date of this phone call with the subject will be recorded as the date of the end of the study of the subject.

If the investigator cannot reach the subject who has previously completed V17 by phone at the end of the Safety Follow-Up Period in Week 56 after a reasonable number of attempts, the date of the last contact (e.g., last visit of the subject, last phone call with the subject) will be recorded as the date of the end of the study of the subject.

The individual end of the study for a subject who has previously completed V17 is defined as V18.

The individual end of the study for a subject who has been discontinued from the study prematurely (early termination) is defined as the date of the early termination of the subject plus 28 days of the Safety Follow-Up Period.

The EOS of the entire study is the end of the Safety Follow-Up Period of the last subject.

Study Population and Main Criteria for Inclusion/Exclusion:

Female or male currently smoking, healthy adult subjects meeting the following main criteria without any restriction on race and ethnicity:

Inclusion criteria:

- Subject is aged from 30 to 65 years old (inclusive).
- Positive urine cotinine test at both screening and V2 (cut-off ≥ 200 ng/mL).
- Has smoked for at least the last 10 years prior to screening.
- Smokes ≥ 10 cigarettes/day on average over the past year prior to screening and V2 as reported by the subject.
- Is willing to quit smoking within the next 30 days, as assessed by the Prochaska's stages of change questionnaire.

Exclusion criteria:

- Clinically relevant gastrointestinal, renal, hepatic, neurological, hematological, endocrine, oncological, urological, pulmonary, immunological, psychiatric or cardiovascular disorders or any other conditions that in the opinion of the investigators would jeopardize the safety of the participant or affect the validity of the study results.
- Abnormal findings on physical examination, in the medical history, or in clinical laboratory results deemed clinically relevant by investigators (as per the common terminology criteria for adverse events [CTCAE]).
- Acute illness (e.g., upper-respiratory-tract infection, viral infection etc.) requiring treatment within 42 days prior to enrolment in the study.

- Use of any prescribed or over-the-counter systemic medication listed in Table 1 within the last 42 days prior to enrolment in the study (except for vitamins, hormonal contraceptives and hormone-replacement therapy).
- The subject has (FEV_1/FVC) < 0.7 and FEV_1 < 80% predicted value at post-bronchodilator spirometry.
- The subject has (FEV_1/FVC) < 0.75 (post-bronchodilator) and reversibility in FEV_1 (that is both > 12% and > 200 mL from pre- to post-bronchodilator values).
- Pregnant or breast-feeding female.

Investigational Product; Dose; and Mode of Administration:

No investigational product will be used in this study.

Duration of Study:

The maximum total duration of the study for a subject will be 66 weeks. A Screening Period of up to 42 days will be followed by V2, plus a period of a maximum of 28 days until the AQD including the TQD visit and the Grace Period, followed by a 52-week smoking abstinence period in an ambulatory setting and a 28-days Safety Follow-Up Period ending after a phone contact with successful quitters in Week 56 (V18 (±3 days)). The EOS of the entire study is the end of the Safety Follow-Up Period of the last subject.

Statistical Methods:

Descriptive statistics (number of subjects [n], number and percent of subjects with missing data, the arithmetic mean, arithmetic standard deviation (SD), median, first and third quartiles, minimum and maximum for continuous data, including geometric mean and coefficient of variation (CV) for data analyzed in the log scale; frequency counts and percentages for categorical data) will be presented overall and at each time point, where applicable. BoExp data will be analyzed in the logarithmic scale and summary estimates will be back-transformed to provide results in the original scale.

Descriptive analysis will be conducted in the population of continuous abstainers with no major protocol deviations. In subjects failing to abstain from smoking, only endpoint data assessed prior to the date of relapse will be included in the analysis.

Descriptive statistics will be provided for endpoint parameters and baseline characteristics of the population retained in the study at each assessment time point. Summary statistics of endpoints will also include the mean change from baseline, together with 95% confidence

interval (CI). Absolute and percent change from baseline will be summarized for endpoints analyzed in the real and logarithmic scale, respectively.

Exploratory Analysis

For the clinical risk endpoints, the smoking abstinence (SA) effect over time will be predicted by a model of the change from baseline adjusted for relevant population characteristics. The pattern and form of the change over time will be modeled using repeated measurement random-effects models. Sex, age, region, and other baseline variables will be considered for inclusion in the model using a backward elimination approach. Goodness of fit will be evaluated by the analysis of residuals (model diagnostics) and by comparing the values predicted versus the observed parameter changes (calibration). Least square estimates of the effect of variables included in the model and the intercept will be reported together with the 95% CI.

Safety Analysis

Adverse event data will serve as the primary assessment of safety. Safety data will be listed and tabulated for the enrolled population.

Sample Size:

Smokers willing to quit smoking within the next 30 days will be enrolled in this study. Enrolment will be allowed until approximately 950 subjects successfully abstain from smoking for at least two weeks after AQD. Unsuccessful quitters (*i.e.*, subjects that smoke after the AQD) will be discontinued from the study.

The sample size of this study is based on our current understanding of the effect and variability of SC from the results of the Lung Health Study [1] in the subject intending to quit smoking at Baseline, since no data on the attrition rate at 12 months after 2 weeks of smoking cessation are available.

In particular, approximately 190 subjects are needed to estimate the mean increase from Baseline of 1.98 [% pred.] [1] in FEV_1[2] at V17, with a 90% probability of obtaining a margin of error (95% CI) of at most ±1 [% pred.]. The anticipated SD of the change from Baseline of 6.4 [% pred.] was estimated using the results of the Lung Health Study [1] and includes an inflation of 10% in order to account for additional sources of variability, including the

[2] Sample size calculations are driven by FEV_1. Other endpoints are assumed to exhibit a larger effect size than FEV_1 and thus would result in smaller sample size estimates.

multi-national nature of the study. As the primary population for analysis will be successful quitters, this sample size was increased to 950, to account for a predicted continuous abstinence rate of 20% expected at week 52 for subjects that successfully abstain from smoking for at least two weeks after. This is based on an assumed increase of 5% from the 15% abstinence rate at week 52 expected for subjects at enrolment [2].

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LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviations

1-NA	1-aminonaphthalene
1-OHP	1-hydroxypyrene
2-NA	2-aminonaphthalene
3-HPMA	3-hydroxypropylmercapturic acid
4-ABP	4-aminobiphenyl
8-epi-$PGF_{2\alpha}$	8-epi-prostaglandin F2α
11-DTX-B2	11-dehydrothromboxane B2
AE	Adverse event
AP	Alkaline phosphatase
Apo A1	Apolipoprotein A1
Apo B	Apolipoprotein B
ATS/ERS	American Thoracic Society/European Respiratory Society
AQD	Actual quit date
B[a]P	3-hydroxybenzo(a)pyrene
BMI	Body mass index
BoExp	Biomarker of exposure
BS	Behavioral support
BUN	Blood urea nitrogen
CC	Conventional cigarette
CEMA	2-cyanoethylmercapturic acid
CI	Confidence interval
CO	Carbon monoxide
COHb	Carboxyhemoglobin
COPD	Chronic obstructive pulmonary disease
CRF	Case report form

CRO	Contract research organization
CSR	Clinical study report
CTCAE	Common terminology criteria for adverse events
CTMS	Clinical trial management system
CV	Coefficient of variation
CVD	Cardiovascular disease
CYP2A6	Cytochrome P450 2A6
D*n*	Day *n* (n^{th} study day)
DMP	Data management plan
ECG	Electrocardiogram
EOS	End of study
ERS	Exposure response study
FDA	Food and Drug Administration
FEF 25-75	Forced expiratory flow 25-75
FEV_1	Forced expiratory volume in one second
FRC	Functional residual capacity
FTND	Fagerström test for nicotine dependence
FVC	Forced vital capacity
GCP	Good Clinical Practice
GGT	Gamma-glutamyl transferase
HbA1c	Glycosylated hemoglobin
HDL-C	High density lipoprotein-cholesterol
HEMA	2-hydroxyethylmercapturic acid
HIV	Human immunodeficiency virus
HMPMA	3-hydroxy-1-methylpropylmercapturic acid
HPHC	Harmful and potentially harmful constituent
Hs-CRP	High sensitivity C-reactive protein
IC	Inspiratory capacity

ICF	Informed consent form
ICH	International Conference on Harmonisation
IEC	Independent Ethics Committee
IRB	Institutional Review Board
LDH	Lactate dehydrogenase
LDL-C	Low density lipoprotein-cholesterol
LLOQ	Lower limit of quantification
LOQ	Limit of quantification
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume
MedDRA	Medical Dictionary for Regulatory Activities
MHBMA	Monohydroxybutenylmercapturic acid
MPO	Myeloperoxidase
MRTP	Modified risk tobacco product
n	Number of subjects
Neq	Nicotine equivalents
NNAL	4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol
NNN	N-nitrosonornicotine
NNK	4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone
NRT	Nicotine replacement therapy
NSAIDs	Non-steroidal anti-inflammatory drugs
o-tol	o-toluidine
PFT	Pulmonary function testing
PMI	Philip Morris International
RBC	Red blood cell (count)
SA	Smoking abstinence
SAE	Serious adverse event

SAP	Statistical analysis plan
S-BMA	S-benzylmercapturic acid
SC	Smoking cessation
SD	Standard deviation
SES	Socio-economic status/situation
ILM	Investigator Laboratory Manual
sICAM-1	soluble inter-cellular adhesion molecule-1
SOP	Standard operating procedure
S-PMA	S-phenylmercapturic acid
SRO	Subject report outcome
TLC	Total lung capacity
TQD	Target quit date
UBC	United BioSource Corporation
ULOQ	Upper limit of quantification
V*n*	Visit *n*
VAS	Visual analog scale
VC	Vital capacity
WBC	White blood cell (count)
WHO	World Health Organization

Explanation of Terms

The following special terms are used in this protocol:

Baseline	Baseline Visit is defined as V2.
Baseline value	Unless specified, baseline is defined as the last available value prior to TQD or AQD, whichever comes first.
Actual quit date	The AQD is the date recorded in the source document on which the subject actually quits smoking and from which onwards total SA is expected. AQD corresponds to the first day without any tobacco/nicotine use (except NRT).
End of study	The individual end of the study for a subject who has previously completed V17 is defined as V18. The individual end of the study for a subject who has been discontinued from the study prematurely (early termination) is defined as the date of the early termination of the subject plus 28 days of the Safety Follow-Up Period. The EOS of the entire study is the individual end of the study of the last subject.
Lost to follow-up (date)	When the PI(s) or designee(s) declare(s) a subject is lost to follow-up, the lost to follow-up date will be recorded and will correspond to the date of the end of study of the subject. If the site has lost track of the subject but the subject has reached the maximum number of study days (465 days), then the PI(s) or designee(s) will declare the subject lost to follow-up at this date.
Conventional cigarette (CC)	The term ‘conventional cigarette’ refers to commercially available cigarettes (manufactured and hand-rolled) and excludes cigars, pipes, bidis, and other nicotine-containing products.
Nicotine replacement therapy	The NRT consists of nicotine-containing products such as nicotine gum, lozenge, patches, inhaler or nasal spray. The NRT should be used as per country label for up to 3 months (+2 weeks) after the start date of NRT. NRT may be started at any time between the TQD and 1 week after the AQD.

Target quit date	Date from which the smoker intends to quit smoking and may start treatment with his/her preferred NRT as per country label, if any and where applicable.
Study completion	The study will be completed once the last successful quitter has reached V18.
Successful quitter	A successful quitter is defined as a subject that was continuously abstinent from smoking from AQD to V17, as assessed by the four criteria described in section 6.2.
Unsuccessful quitter	An unsuccessful quitter is defined as a subject that was not continuously abstinent from smoking from AQD to V17, as assessed by the four criteria described in section 6.2.

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 form [ICF], subject information sheet, subject recruitment procedures [*e.g.*, advertisements], written information to be provided to the subjects, available safety information, the Investigator's and designee curriculum vitae and/or other evidence of qualifications and any other documents requested by an Institutional Review Board [IRB] or Independent Ethics Committee [IEC]), will be submitted for review and approval to the relevant IRB/IEC. The IRB/IEC shall be appropriately constituted and perform its functions in accordance with the International Conference on Harmonisation (ICH) Tripartite Guidance for Good Clinical Practice (GCP) and local requirements, as applicable.

Where applicable, and in accordance with GCP and 21 CFR part 56, a written confirmation of the IRB approval should be provided to the Sponsor. This should identify the study (Principal Investigator and designee's name, study number, and title) and the documents that have been approved by the IRB, with dates and version numbers, as well as the date of approval. The composition of the IRB, 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 the Investigator file, and a copy will be filed in the study master file at the Sponsor or 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 Investigator. 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 designee or by the Sponsor in order to eliminate immediate hazards to the subjects. If such a change to the protocol is felt to be necessary by the Investigator or designee, 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. Separate ICFs will be signed by the subject for the collection and storage of bio-banking 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 is consistent with applicable regulatory principles of ICH/GCP.

The Investigator or designee 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 [3] 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 atV1, the Investigator or designee 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 or the designee 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 discontinue his/her participation at any time.

Once the subject has received all the necessary information, and if he/she agrees to participate, this will be documented in the ICF which includes both the subject information sheet and informed consent by the date and time personally signed and signature of both the subject and the person who conducted the inform consent discussion during the visit at V1. No study-specific procedures will be performed before the ICF has been signed (including date and time).

The signed and personally dated original and completed ICF(s) must be kept by the Investigator and filed in the Investigator study 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 discontinues from the study, the data collected until the point of discontinuation will be maintained as part of the study data and the samples collected prior to discontinuation may be analyzed, unless he/she refuses in writing.

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 analysis performed will be covered by data confidentiality, as for the main analysis described in this protocol.

1.3.2 Informed Consent Form for Long-term Bio-Banking

Separate ICFs will be signed and personally dated by the subject for the collection of samples and their long-term bio-banking storage. The subject's participation in the study does not depend on his/her consent to these separate ICFs.

- One separate ICF to obtain consent for serum/plasma and urine collection and long-term storage for subsequent analysis of biomarkers of exposure (BoExp) and clinical risk endpoints following completion of this study. No genetic, transcriptomics and/or lipidomics testing will be done on these samples.
- One separate ICF to obtain consent for collection and long-term storage of blood/ plasma samples for further transcriptomics and lipidomics analyses.

Transcriptomics, lipidomics and molecular analyses will provide insight into the biological processes that take place following smoking cessation (SC).

Each subject will be given full and adequate oral and written information about the nature, purpose, possible risks and benefits of bio-banking, and the Investigator 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 at any time. Once the subject has received all the necessary information, and if he/she agrees to participate, this will be documented by the date and time personally signed and signature of both the subject and the personnel who conducted the informed consent discussion. The subject's consent to collection of any samples for long-term storage in a bio-bank is not a requirement for his/her participation in the study (section 1.3.1).

1.3.3 Amendment to the Informed Consent Form

If a protocol amendment is required, an amendment may be required to the ICF. If revision of the ICF is necessary, the Investigator or designee will, with the support of the Sponsor, ensure that the documents have been reviewed and approved by a relevant IRB/IEC before subjects are informed and sign and personally date the amended 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, and Investigator and designee abide by the principles of the ICH guidelines on GCP. These guidelines apply specifically to pharmaceutical development, but nevertheless provide a robust and ethical framework for conducting a clinical study on smoking cessation. The study will also be conducted in accordance with the general ethical principles outlined in the Declaration of Helsinki [3].

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

2 INTRODUCTION

2.1 Background

Cigarette smoking causes pulmonary diseases, cardiovascular diseases (CVD) and other serious diseases in smokers [4]. There is no safe cigarette, and the best way for smokers to reduce the adverse health consequences of smoking is to quit. Despite the risks which are attributable to smoking, some smokers cannot refrain from smoking or decide to continue smoking. For those smokers who are not able or not willing to quit, Philip Morris International (PMI) is developing alternative approaches by developing products with the potential to reduce the risks of tobacco-related diseases. These products are referred by the Food and Drug Administration (FDA) as modified risk tobacco products (MRTP) [5].

The disorders induced by conventional cigarettes (CC) smoking are complex, as there are multiple causal chains, and tobacco-related diseases develop over many years. The exposure to harmful and potentially harmful constituents (HPHCs) contained in cigarette smoke affects multiple organ systems, disease pathways, and mechanisms such as inflammation, oxidative stress, platelet activation and lipid metabolism which occur simultaneously. It is well-known that there is a dose-response relationship between cigarette smoke exposure and smoking-related diseases such as chronic obstructive pulmonary disease (COPD), CVD and lung cancer [6, 7]. However, there is no single clinical risk endpoint or biomarker that is considered as a validated surrogate measure reflecting the biological process, physiological system, and/or a mechanism of action that is associated with or known to contribute to smoking-related diseases.

The US Institute of Medicine (IOM) refers to smoking cessation as the “gold standard” for the assessment of a reduced risk products (RRP), providing “an aspirational goal for risk and exposure.” [6].

It has been shown that several functional and biological markers (clinical risk endpoints) associated with smoking-related adverse health effects are favorably changed after SC in both the short and long term [8-10]. These effects are sufficiently sensitive to changes in smoking status to suggest that they can be used for the risk assessment of candidate MRTPs. Thus, conventional cigarette (CC) smoking and SC are considered natural benchmarks for assessing the exposure and risk reduction potential of candidate MRTPs [5, 11]. The observation of clinical risk endpoints after switching from CC to a candidate MRTP in comparison to SC should be a reasonable indicator for disease risk reduction to the individual smoker. The clinical risk endpoints selected for this study are the markers with the strongest scientific evidence of favorable changes following SC.

2.2 Purpose of the Study

There is sufficient literature reporting favorable changes in clinical risk endpoints with SC, however the observation time period of one year of continuous abstinence is often sparse or even not available, and the currently available information is derived from multiple clinical studies making it difficult to compare the findings.

The primary objective of the SCR Study is to establish data on changes on a broad range of BoExp and Clinical Risk Endpoints as observed after 12 months of smoking cessation.

This study will serve two purposes: 1) to be used as a benchmark in the context of candidate MRTP assessments (*i.e.,* to allow comparison between the risks and benefits from the use of a candidate MRTP compared to SC), and 2) corroborate and supplement the existing data on biological and functional health effects of SC.

The primary objective clearly differentiates the SCR study from other Smoking Cessation studies where testing the efficacy of a smoking cessation treatment (i.e. drug or behavioral cessation support) is the primary objective.

2.3 Anticipated Benefits and Risks

2.3.1 Anticipated Benefits

Extensive SC support (SC counseling, behavioral support (BS) and training on the use of nicotine replacement therapy (NRT) will be provided over the duration of the study. The combination of these different methods of smoking cessation support has been shown to markedly increase the success rate of quitting smoking. Subjects who participate in this study will also benefit from repeated and extensive health check-ups, which may help to uncover undiagnosed medical conditions.

2.3.2 Anticipated Foreseeable Risks due to Study Procedures

The risk 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.

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.

The subject will use NRT, if any, as per the label approved in the subject's country of residence. The risk related to the use of NRT is described in detail in the NRT label leaflet and will be explained to the subjects by the Investigator or designee. The possibility of unforeseeable events/risks due to study related procedures will be explained at V1. Mitigation of such risks will include close monitoring and medical supervision to detect any unforeseeable risk or safety signals at the earliest time possible.

3 STUDY OBJECTIVES AND ENDPOINTS

The objectives and endpoints of this study are:

1. To describe the clinical, biological and functional changes in smokers who are continuously abstinent from smoking.

   Clinical risk endpoints associated with CVD at week 13 (V8), week 26 (V11) and week 52 (V17):

   - White blood cell count (WBC), platelet count, glycosylated hemoglobin (HbA1c), and carboxyhemoglobin (COHb) in blood.
   - High and low density lipoprotein cholesterol (HDL-C, and LDL-C), myeloperoxidase (MPO), soluble intercellular adhesion molecule-1 (sICAM-1), apolipoprotein A1 and B (Apo A1 and Apo B), and high sensitivity C-reactive protein (hs-CRP) in serum.
   - Fibrinogen, and homocysteine in plasma.
   - Albumin, 11-dehydrothromboxane B2 (11-DTX-B2) and 8-epi-prostaglandin-alpha (8-epi-$PGF_{2\alpha}$) in urine (expressed as concentrations adjusted to creatinine).

   Clinical risk endpoints associated with respiratory diseases:

   - Spirometry (pre and post-bronchodilator): Forced expiratory volume in 1 second (FEV_1), forced vital capacity (FVC), FEV_1/FVC, forced expiratory flow (FEF 25-75), at week 13 (V8), week 26 (V11) and week 52 (V17).
   - Lung volume: vital capacity (VC), total lung capacity (TLC), functional residual capacity (FRC), inspiratory capacity (IC), at selected sites specialized for lung function testing at week 13 (V8), week 26 (V11) and week 52 (V17).
   - Cough symptoms (intensity and frequency), amount of sputum production and bothersomeness of cough symptom from the cough questionnaire at week 13 (V8), week 26 (V11) and week 52 (V17).

   Clinical risk endpoint associated with xenobiotic metabolism at week 13 (V8), week 26 (V11) and week 52 (V17):

   - Cytochrome P450 2A6 (CYP2A6) activity: Molar metabolic ratio of *trans*-3-hydroxycotinine/cotinine in plasma.

Clinical risk endpoint associated with genotoxicity at week 13 (V8), week 26 (V11), and week 52 (V17):

- Total 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (Total NNAL) in urine (expressed as concentration adjusted to creatinine).

2. To describe the changes in BoExp to harmful and potentially harmful constituents (HPHCs) in smokers who are continuously abstinent from smoking.

   BoExp to HPHCs at week 13 (V8), week 26 (V11) and week 52 (V17):

   - BoExp to CO: CO in exhaled breath (expressed as ppm).*
   - BoExp to nicotine: cotinine and nicotine in plasma and nicotine equivalents (Neq) in urine[3]*
   - BoExp to 1,3-butadiene : monohydroxybutenylmercapturic acid (MHBMA).*
   - BoExp to acrolein: 3-hydroxypropylmercapturic acid (3-HPMA).*
   - BoExp to acrylonitrile: 2-cyanoethylmercapturic acid (CEMA).*
   - BoExp to benzo(a)pyrene: 3-hydroxybenzo(a)pyrene (B[a]P).*
   - BoExp to pyrene: Total 1-hydroxypyrene (Total 1-OHP).*
   - BoExp to crotonaldehyde: 3-hydroxy-1-methylpropylmercapturic acid (3-HMPMA).*
   - BoExp to N-nitrosonornicotine: total N-nitrosonornicotine (Total NNN).*
   - BoExp to 4-aminobiphenyl: 4-aminobiphenyl (4-ABP).
   - BoExp to benzene: S-phenylmercapturic acid (S-PMA).
   - BoExp to 1-aminonaphthalene: 1-aminonaphthalene (1-NA).
   - BoExp to 2-aminonaphthalene: 2-aminonaphthalene (2-NA).
   - BoExp to o-toluidine: o-toluidine (o-tol).
   - BoExp to ethylene oxide: 2-hydroxyethylmercapturic acid (HEMA).
   - BoExp to toluene: S-benzylmercapturic acid (S-BMA).

[3] Nicotine equivalents (Neq) are defined as molar sum of free nicotine, nicotine-glucuronide, free cotinine, cotinine-glucuronide, free *trans*-3'-hydroxycotinine, *trans*-3'- hydroxycotinine-glucuronide.

All BoExp measured in urine, will be expressed as concentrations adjusted to creatinine. Only BoExp marked with " * " will be assessed at week 26 (V11) and week 52 (V17).

3. To describe the rate of continuous SA at each visit following the actual quit date (AQD) of smoking cessation.

4. To monitor the safety:

   - Adverse events (AEs)/ serious adverse events (SAEs).
   - Body weight.
   - Vital signs.
   - Spirometry
   - Electrocardiogram (ECG).
   - Clinical chemistry, hematology and urine analysis safety panel.
   - Physical examination.
   - Concomitant medications.

Additional study assessments:

- Prochaska 'Stage of Change' questionnaire.
- Fagerström test for nicotine dependence (FTND, revised version).
- Socio-economic status (in the following countries: US, UK, Poland, Germany and Japan).
- Lifestyle assessments.

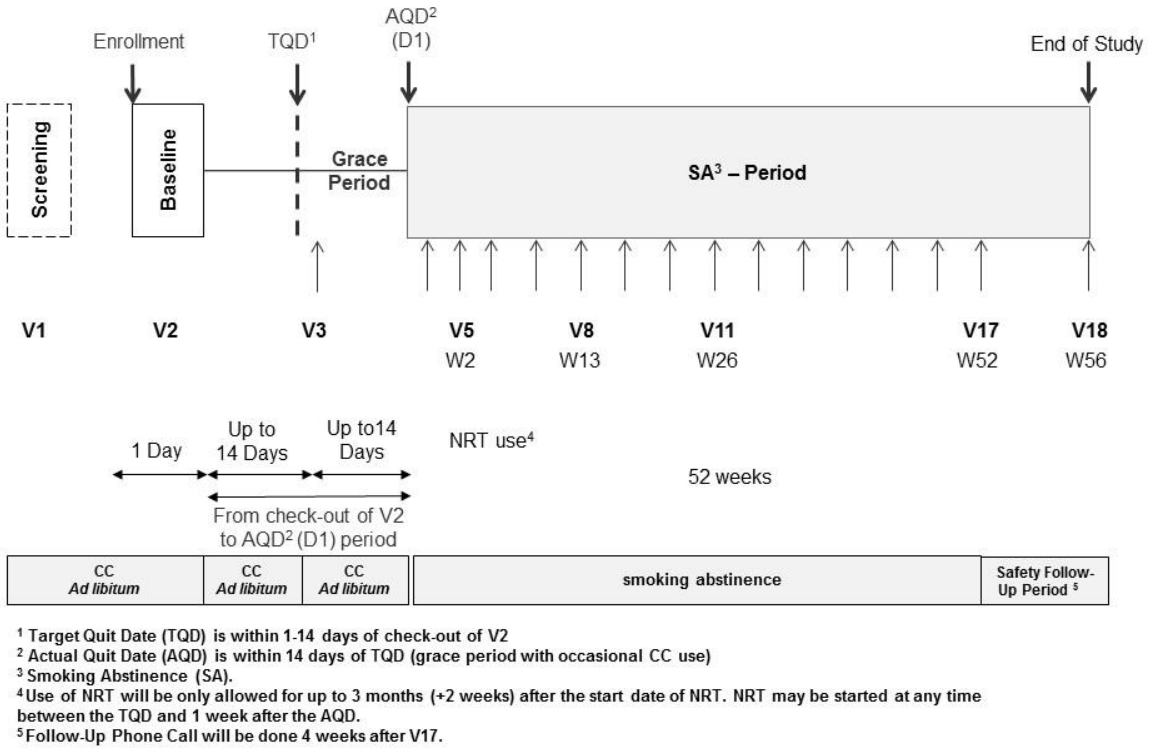
4 INVESTIGATIONAL PLAN

4.1 Overall Study Design and Plan

This multi-region, multi-center, ambulatory study will be conducted in the US, Japan and Europe. Smokers who are motivated to quit smoking within the next 30 days at the Screening Visit will be enrolled to reach approximately 950 subjects continuously abstinent from smoking from AQD onwards at week 2 (V5), in order to achieve at least 190 successful quitters expected to complete the study. Once approximately 950 subjects will reach V5, screening and enrolment will be stopped. Subjects already enrolled in the study at that point in time and that are between V2 and V5, will be kept in the study and allowed to progress over Visit 5. On the contrary, on-going subjects that have completed screening visit and are before visit 2 will be discontinued from the study and classified as screen failures.

Smokers who are not continuously abstinent from smoking (*i.e.,* free from tobacco product use (*e.g.*, CC, pipes, cigars, snus) or any nicotine containing product (including electronic cigarettes) other than NRT)) from their AQD onwards will be discontinued from the study (see sections 5.3 and 6.2).

NRT use will be allowed as per label for up to 3 months (+2 weeks) after the start date of NRT to support the subject to remain abstinent from smoking. NRT may be started at any time between the TQD and 1 week after the AQD. The start day of NRT will be counted as Day 1 of NRT use. From that day on, the duration of NRT use must not exceed 3 months + 2 weeks. NRT will be reimbursed.



[1] Target Quit Date (TQD) is within 1-14 days of check-out of V2
[2] Actual Quit Date (AQD) is within 14 days of TQD (grace period with occasional CC use)
[3] Smoking Abstinence (SA).
[4] Use of NRT will be only allowed for up to 3 months (+2 weeks) after the start date of NRT. NRT may be started at any time between the TQD and 1 week after the AQD.
[5] Follow-Up Phone Call will be done 4 weeks after V17.

Abbreviations: CC = Conventional cigarettes; NRT = Nicotine replacement therapy; SA = Smoking abstinence; V = Visit; W = week

Figure 2 Study Design

Screening Period

The Screening Period will take place 1 to 42 days prior to enrolment at V2 (Baseline). Eligibility of the subjects to participate in the study will be assessed during the Screening Visit (V1). Subjects who are still eligible at the end of V1 will be provided with urine containers and instructions for the 24-hour urine home collection. Eligible subjects will be contacted prior to V2 to confirm their eligibility and to take an appointment for V2.

Baseline Visit - V2 (from check-in to check-out from the site)

Twenty-four-hour urine collection will start in the morning of the day prior to V2 and end 24 hours later in the morning of V2. Enrolment of the subject will take place after the subject's check-in on site with the cooled urine container(s) filled with his/her 24-hour urine and re-check of inclusion criteria n°4 and n°6. All other procedures and data collection will be completed following the enrolment of the subject. All subjects will continue smoking their preferred brand of CC.

Before check-out, subjects will be asked to define their target quit date (TQD), the date from which the subject will stop smoking. The TQD must be within 14 days after V2.

From Check-out of V2 to Actual Quit Date

This period aims to identify subjects who are more motivated and more likely to quit as well as to remain continuously abstinent from smoking for the whole duration of the study. The period starts from check-out of V2 and ends with the actual quit date (AQD) (Day 1) including the TQD, V3 and the Grace Period. This period might last up to 28 days for each subject.

The subject will be asked to come to the clinic for V3 within 24 to 48 hours after their defined TQD. The goal of this visit will be to ensure that the subject has actually quit smoking and to provide him/her with the necessary support. From V3 onwards, SC counseling and BS will be provided to the subject according to the SC support plan. Additional SC support will be offered at any time when requested by the subject.

A Grace Period of a maximum of 14 days will be allowed after the TQD, during which occasional slips (defined as occasional use of nicotine and/or tobacco-containing products day) of smoking will be accepted. From the AQD onwards, strict abstinence from any tobacco- or nicotine-containing product (including electronic cigarettes) other than NRT is required. Subjects will be asked to record their AQD and to communicate this date to the site in order to schedule following visits. The latest possible day for the AQD to occur is defined as the last day of the Grace Period (i.e., TQD + 14 days).

The Smoking Abstinence Period (from the AQD up to the check-out of V17 [week 52])

From the AQD, subjects will be asked to come on site for at week 1 (V4), at week 2 (V5) and then on a monthly basis at week 4 (V6), at week 9 (V7), at week 13 (V8), at week 17 (V9), at week 22 (V10), at week 26 (V11), at week 30 (V12), at week 35 (V13), at week 39 (V14), at week 43 (V15), at week 48 (V16) , and at week 52 (V17). Visits will be scheduled based on the AQD. A time window of ± 8 days is allowed for the visits, with the exception of V4 (± 3 days) and V5 (± 3 days).

The V8 , V11 , and V17 will correspond to full assessment visits at site(s) where 24-hour urine and blood sampling will be collected for analysis of BoExp and clinical risk endpoints. The

collection of the 24-hour urine will start at home in the morning the day before the visit and will end 24 hours later in the morning of the day of the visit to the clinic.

The Safety Follow-Up Period and Phone Contact (28 days after the check-out of V17 [V18 (±3 days); week 56])

A subject who has completed V17, or a subject who has been discontinued from the study prematurely (early termination), will enter a 28-days Safety Follow-Up Period during which spontaneously reported new AEs/SAEs will be recorded, and the active follow-up of ongoing AEs/SAEs will be done by the site. All AEs will be followed-up until resolved, stabilized (i.e., no worsening of the event), or a plausible explanation for the event has been found until the end of the Safety Follow-Up Period.

At the end of the Safety Follow-Up Period in Week 56 (V18 (±3 days)), the investigator will attempt to contact only the subject who has previously completed V17 by phone to check if all AEs/SAEs potentially occurring during the Safety Follow-Up period are fully reported and for self-reporting by the subject on continuous smoking abstinence. At the end of the Safety Follow-Up Period, all ongoing AEs will be documented as "ongoing" and will not be followed-up by the Investigator. At the discretion of the Investigator, the subject will be referred to his/her General Practitioner for follow up on ongoing AEs.

If the investigator can reach the subject who has previously completed V17 by phone at the end of the Safety Follow-Up Period in Week 56, the date of this phone call with the subject will be recorded as the date of the end of the study of the subject.

If the investigator cannot reach the subject who has previously completed V17 by phone at the end of the Safety Follow-Up Period in Week 56 after a reasonable number of attempts, the date of the last contact (e.g., last visit of the subject, last phone call with the subject) will be recorded as the date of the end of the study of the subject.

The individual end of the study for a subject who has previously completed V17 is defined as V18.

The individual end of the study for a subject who has been discontinued from the study prematurely (early termination) is defined as the date of the early termination of the subject plus 28 days of the Safety Follow-Up Period.

The EOS of the entire study is the end of the Safety Follow-Up Period of the last subject.

4.2 Rationale for Study Design

This clinical study aims at understanding the clinical, biological and functional changes after one year of smoking cessation in healthy subjects. Healthy subjects will be included in this study, since the impact of potential diseases on the study endpoints is difficult to evaluate.

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.

This study is of non-investigational nature and will be used to establish a benchmark to compare the risks and benefits for a smoker switching from a CC to a candidate MRTP compared to smoking cessation. No control arms are required.

Since literature [12] indicates that > 40% of subjects who remain tobacco free at 2 weeks continue to remain tobacco free for > 6 months, whereas > 80% of those who relapse in the first 2 weeks are smoking at 6 months, a period starting from check-out of V2 and ending with the V5 including the TQD, V3, the Grace Period and AQD has been implemented in the study design to evaluate smoking abstinence status and to select the subjects who are more likely to remain smoking abstinent for the whole duration of the study. Subjects discontinued from the study between AQD and V5 will be replaced in order to achieve approximately 950 subjects who are continuously abstinent from smoking at V5. From V5 onwards, subjects will not be replaced.

To help smokers quitting tobacco, subjects will be provided with SC support and NRT (if requested by the subject). NRT are recommended as SC aids in Europe [13], Japan [14] and US [15], in addition to SC support. Other drugs to aid SC have been approved in several countries (*e.g.*, bupropion and varenicline). However, since information on their effect on the selected clinical risk endpoints is sparse, their use will not be allowed in this study.

Twenty-four hour-urine that will be collected in this study is the standard method to measure the levels of excretion of BoExp.

As part of the characterization of the study population it is important to measure variables that have been shown to be related to nicotine dependence and product reinforcing value. Based on prior tobacco research these factors include age, gender, ethnicity, educational and socio-economic status (SES), tobacco use history, expectations of the effects of the products tested, nicotine exposure, health and mental health status and use of psychoactive substances. In order to capture such data and allow comparing populations across studies, subjects will be asked questions about their socio-economic status.

4.3 Appropriateness of Measurements

The clinical risk endpoints to be assessed are selected based on a) their association with smoking-related diseases b) their association with smoking status, c) their reversibility upon SC/abstinence, and d) their suitability to be measured with valid and robust methods in clinical studies, e) timeframe of reversibility of measure in the perspective of the study duration. These clinical risk endpoints are associated with cardiovascular and respiratory diseases, genotoxicity and xenobiotic metabolism as presented in Appendix 4.

The BoExp (list in Appendix 5) measured in this study were selected based on the following criteria: 1) HPHCs to be assessed in this study are derived from the list of HPHCs recommended for lowering in cigarette smoke as defined by the World Health Organization (WHO [16] and the draft guidance on Reporting Harmful and Potentially Harmful Constituents in Tobacco Products and Tobacco Smoke [17]; 2) the HPHC should be specific to the source of exposure with other sources being minor or non-existent; 3) the BoExp to an HPHC should be easily detectable using reliable, reproducible, precise analytical methods; 4) the HPHC should reflect a specific toxic exposure or be a reliable surrogate of exposure to HPHCs; 5) The list of HPHCs should include HPHCs from both gas and particulate phase; 6) The list of HPHCs should include a broad variety of chemical classes and organ toxicity classes (carcinogen, cardiovascular toxicant, respiratory toxicant, reproductive and development toxicant, addiction potential) and 7) represent HPHCs formed at different temperature levels.

All questionnaires utilized for this study, except the cough-VAS and the socio-economic questionnaire, are available as validated questionnaires.

4.4 Study Duration

The maximum total duration of the study for a subject will be 66 weeks. A Screening Period of up to 42 days will be followed by V2, plus a period of a maximum of 28 days until the AQD including the TQD visit and the Grace Period, followed by a 52-week smoking abstinence period in an ambulatory setting and a 28-days Safety Follow-Up Period ending after a phone contact with successful quitters in Week 56 (V18 (±3 days)). The EOS of the entire study will be defined as the end of the Safety Follow-Up Period of the last subject.

5 STUDY POPULATION

5.1 Selection of Study Population

Smoking adults (female or male), healthy subjects with no restriction on race and ethnicities, who have smoked at least 10 CC per day on average for the last 12 months and who have been smoking for at least the last 10 years will be enrolled in this study.

The study will be a multi-center study, with approximately 50 sites located in Europe, Japan and US.

5.1.1 Inclusion Criteria

Each subject enrolled at Baseline Visit (V2) must meet the following criteria:

Inclusion Criteria	Screening (V1)	Baseline (V2)
1. Subject has signed the ICF and is able to understand the information provided in the ICF.	X	
2. Subject is aged from 30 to 65 years old (inclusive).	X	
3. Smoking, healthy subject as judged by the Investigator based on all available assessments from the Screening Period (*e.g.*, safety laboratory, spirometry, vital signs, physical examination, ECG, concomitant medications and medical history).	X	
4. Subject smokes at least 10 commercially available CCs per day on average (no brand restrictions), for the last 12 months, based on self-reporting. Furthermore, the subject has been smoking for at least the last 10 years prior to screening. The smoking status will be verified based on a urinary cotinine test (cotinine ≥ 200 ng/mL).	X	X
5. The subject is willing to quit smoking within the next 30 days, as assessed by the Prochaska's 'Stage of Change' questionnaire.	X	
6. The subject is ready to comply with the study protocol (*e.g.*, readiness to accept continuous smoking abstinence for 52 weeks).	X	X

5.1.2 Exclusion Criteria

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

Exclusion Criteria	Screening (V1)	Baseline (V2)
1. As per the Investigator (or designee) judgment, the subject cannot participate in the study for any reason (e.g., medical, psychiatric and/or social reason).	X	
2. The subject is legally incompetent, physically or mentally incapable of giving consent (e.g., emergency situation, under guardianship, in a social or psychiatric establishment, prisoner or involuntarily incarcerated).	X	
3. Clinically relevant gastrointestinal, renal, hepatic, neurological, hematological, endocrine, oncological, urological, pulmonary, immunological, psychiatric or cardiovascular disorders or any other conditions that in the opinion of the investigators would jeopardize the safety of the participant or affect the validity of the study results.	X	
4. Abnormal findings on physical examination, in the medical history, or in clinical laboratory results deemed clinically relevant by investigators (as per the common terminology criteria for adverse events [CTCAE]).	X	
5. Acute illness (e.g., upper-respiratory-tract infection, viral infection etc.) requiring treatment within 42 days prior to enrolment in the study.		X
6. Use of any prescribed or over-the-counter systemic medication listed in Table 1* (except for vitamins) within the last 42 days prior to enrolment in the study (except for hormonal contraceptives and hormone-replacement therapy).		X
7. The subject has (FEV_1/FVC) < 0.7 and FEV_1 < 80% predicted value at post-bronchodilator spirometry.	X	
8. The subject has (FEV1/FVC) < 0.75 (post-bronchodilator) and reversibility in FEV1 (that is both > 12% and > 200 mL from pre- to post-bronchodilator values).	X	
9. The subject has a body mass index (BMI) < 18.5 or ≥ 35 kg/m^2.	X	
10. As per the Investigator's or designee's judgment, the subject has medical conditions which require or will require in the course of the study, a medical intervention (e.g., start	X	

Exclusion Criteria	Screening (V1)	Baseline (V2)
of treatment, surgery, hospitalization) which may interfere with the study participation and/or study results.		
11. The subject has a positive alcohol test and/or he/she has a history of alcohol abuse that could interfere with his/her participation in the study.	X	
12. The subject has a positive urine drug test.	X	
13. The subject has positive serology test for human immunodeficiency virus (HIV)1/2, Hepatitis B or Hepatitis C.	X	
14. The subject has donated or received whole blood or blood products within 3 months prior to V1.	X	
15. The subject has been previously screened for this study.	X	
16. The subject is a current or former employee of the tobacco industry or their first-degree relatives (parent, sibling, child).	X	
17. The subject is an employee of the investigational site or any other parties involved in the study or their first-degree relatives (parent, sibling and child).	X	
18. The subject has participated in a clinical study within 3 months prior to V1.	X	
19. For women only: subject is pregnant (does have positive pregnancy test) or is breast feeding.	X	X

* Concomitant Medication with potential impact on Clinical Risk Endpoints (section 6.4). Subjects using salbutamol for post-bronchodilator spirometry testing at screening will not be excluded from the study.

5.2 Recruitment Strategies and Retention

Stratified sampling will be used to ensure adequate representation of subjects by sex (*i.e.*, to have at least 40% of each sex at enrolment (*see* enrolment plan in a separate document)).

Enrolment and attrition rates will be monitored during the study. In particular, the enrolment strategy and sample size may be adapted, depending on the actual attrition rate observed during the study conduct. If a higher than expected attrition rate is observed, the sample size may be increased to ensure adequate statistical power. If a lower than expected attrition rate is

observed, all subjects continuously abstinent from smoking, as assessed by the four criteria described in section 6.2, will be retained in the study.

Smoking cessation support will be provided to the subject to prevent him/her from smoking during the study (section 1.1).

5.3 Discontinuation of Subjects from the Study

Discontinued subjects will include both subjects who withdraw from the study (subject's decision) or subjects who are removed from the study (*e.g.,* subject who are not continuously abstinent from smoking after AQD, section 6.2). A subject can only be discontinued from the study after enrolment.

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 main study, although they are not obliged to disclose it.

If the subject withdraws his/her consent for the main study (main ICF), the Investigator or designee needs to document if:

- If applicable, the subject still consents for long-term bio-banking.
- Whether the subject requested withdrawal of Health Insurance Portability and Accountability Act (HIPAA) authorization (for the US sites only).
- The subject agrees to undertake the early termination procedures (section 9.6)

This information needs to be fully documented in the source document and CRF.

When a subject is discontinued from the study, he/she will be asked to perform the examination procedures planned in section 9.6 as soon as possible after the time of discontinuation unless the subject refuses to perform the assessments in writing.

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

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

- Withdrawal of informed consent.
- Any AE or condition (including clinically relevant changes in a laboratory parameter) which at the discretion of the Investigator or designee no longer justifies the subject's participation in this study.
- Pregnancy test is positive (section 8.5 for further details on the management of pregnancies).

- The Sponsor, the Investigator or designee terminates the study. If the Sponsor, the Investigator or designee decides to prematurely terminate the study, the subject will be promptly informed. The Investigator or designee should report the fact and the reason of termination in writing to the IRB or IEC.
- Discontinuation is considered to be in the best interest for the subject himself/herself or for other subjects participating to the study, as judged by the investigator or designee.
- Non-compliance with continuous smoking abstinence (see section 6.2), as assessed by the following four criteria:
    1. Self-reporting by the subject of use of any of the following product from AQD onwards:
        - Tobacco product, such as CC, snus, cigars.
        - Nicotine-containing products, other than NRT.
        - NRT continued after the allowed timeframe, i.e. 3 months (+2 weeks) after the start date of NRT.
        - Electronic cigarettes (with or without nicotine).
    2. Smoking abstinence verification by exhaled CO breath test at each visit from V4 onwards (CO breath test is > 10 ppm)
    3. Smoking abstinence verification by urine cotinine test in spot urine at site at each visit from V10 onwards (urine cotinine test is ≥ 100 ng/ml).
    4. Smoking abstinence verification by analysis of the free cotinine concentration (part of the nicotine equivalents) in 24-hour-urine collected at Visit 11 (urine cotinine concentration is ≥ 50 ng/mL).

    If any one of these four criteria is met, the subject must be discontinued.

Subjects may be discontinued from the study for any of the following reasons on the judgment of the Investigator or designee:

- Non-compliance to the study procedures other than compliance to continuous smoking abstinence.
- Use of medication supportive to smoking cessation, other than NRT, from the TQD onwards.

Subjects discontinued from enrolment to V5 will be replaced in order to achieve approximately 950 subjects who are continuously abstinent from smoking at V5. From V5 onwards, subjects

will not be replaced. All subject discontinuations have to be documented properly in the source document and in the CRF.

5.4 Assessments at Visit 17 to be performed if Total NNAL is above the cut-off at Visit 11

Analysis of Total NNAL will be performed by a central laboratory to determine which assessments will be performed at Visit 17. The concentration of Total NNAL will be determined in 24-hour urine collected at Visit 11 (cut-off < 75.9 pg/mL) [18]. If the concentration of Total NNAL is ≥ 75.9 pg/mL, the subject will remain in and continue with the study, unless the subject is discontinued for other reasons. However, for this subject, only the following assessments at Visit 17 will be performed:

- Smoking cessation support
- Prior/Concomitant medication
- Pregnancy test (all female subjects)
- Vital signs
- CO Breath test
- Cotinine test in spot urine (cut-off < 100 ng/mL)
- AE/SAE recording.

5.5 Lost to Follow-up

Reasonable number of attempts to contact the subject (including written correspondence and phone calls) should be done and documented in the source documents by the site. The date of the last contact (e.g. last visit, last phone call) should also be recorded in the source document. When the PI(s) or designee(s) declare(s) a subject is lost to follow-up, the lost to follow-up date will be recorded and will correspond to the date of the end of study of the subject.

If the site has lost track of the subject but the subject has reached the maximum number of study days (465 days), then the PI(s) or designee(s) will declare the subject lost to follow-up at this date.

5.6 Violation of Selection Criteria

Subjects who, after signing the ICF at screening, do not meet the entry criteria at Screening or at V2 or do not come to V2 with their 24h urine container filled will be considered as screen failures. Re-screening will not be permitted.

If a violation of selection criteria is detected, subjects might be discontinued from the study based on a case-by-case decision of the PI.

6 RESTRICTIONS AND SUPPORT DURING THE STUDY

6.1 Smoking Cessation Support

Information on the risk of smoking and SC advice will be provided to the subject. The approach to SC support including SC counseling and behavioral support in this study will be explained and the benefits and risks of using NRT discussed with the subject. The Investigator or designee will provide information on the different NRTs available and the Investigator or designee and the subject will agree on which NRT will be used as supportive medication. The subject may choose to not use NRT and to rely on smoking cessation counseling and behavioral support only.

Once the NRT is selected, the subject will be advised on how to use the NRT as per approved country label and for up to 3 months (+2 weeks) after the start date of NRT. NRT may be started at any time between the TQD and 1 week after the AQD.

If the NRT selected (*e.g.,* gum, lozenge, patch, nasal spray, depending on the availability in the country of study) is not available over the counter, the Investigator or designee will prescribe the selected NRT as per local country regulations. The combined use of NRT (*e.g.,* patch and gum together) or the switch from one NRT to another one will be allowed in this study as per country label (*e.g.,* NRT combined use for EU allowed, but not in JP) and for up to 3 months (+2 weeks) after the start date of NRT. No medication supportive for SC other than NRT (*e.g.,* bupropion or varenicline) will be allowed.

A SC specialist (*e.g.*, psychologist) may be contacted and will be available upon the subject's request, or if considered necessary, upon the request of the Investigator or designee.

Smoking cessation support, including SC counseling and behavioral support, will be provided at each visit and in-between visits throughout the study from the TQD onwards as defined in the SC support plan (separate document) and whenever requested by the subject.

6.2 Compliance

Compliance with continuous smoking abstinence is defined by the four following criteria:

1. No self-reporting by the subject of use of any of the following product from AQD onwards:
   - Tobacco product, such as CC, snus, cigars.
   - Nicotine-containing products, other than NRT.
   - NRT continued after the allowed timeframe, i.e. TQD + 3 months (+2 weeks).
   - Electronic cigarettes (with or without nicotine).

2. CO breath test ≤ 10 ppm [19], as verified at each visit from V4 onwards.
3. Urine cotinine test < 100 ng/mL in spot urine at site, as verified at each visit from V10 onwards [19].
4. Free cotinine concentration < 50 ng/mL in 24-hour-urine collected at Visit 11 [19].

If any of these four criteria is not met, the subject must be discontinued (see section 5.3).

6.3 Restrictions

6.3.1 Smoking Restrictions

Subjects will be allowed to use their own brand of CC until their AQD. Occasional slips of using CC during the Grace Period between TQD and AQD is allowed as described in section 4.1. The use of NRT between the TQD and AQD while no full smoking abstinence is achieved will depend on the NRT country label. From their AQD, use of any tobacco, nicotine containing product other than NRT, as well as e-cigarettes will not be allowed in this study.

At Screening Visit (V1) and V2 pre- and post-bronchodilator spirometry will be done after at least 1 hour of not smoking (section 7.4.7.1).

6.3.2 Dietary Restrictions

Subjects have to fast for 10 hours prior to blood draws for:

- Hematology and clinical chemistry safety laboratory except at the Screening Visit (V1).
- Clinical risk endpoints.
- Serum/plasma/blood bio-banking.

Subjects do not have to fast prior to the timepoints for the urine analysis of the safety panel.

6.4 Concomitant Medication

For the definition of prior and concomitant (ongoing medications), please see section 7.4.3. Medication will be allowed during the study and will carefully be monitored by the Investigator or designee. The Investigator or designee is responsible for the medical care including medication of the subjects during their participation in the study. Any decisions regarding the prescription of medication will be made in the best interest of the subject. Any use of concomitant medication, including NRT, must be fully documented in the source document and recorded into the CRF. For NRT, the Investigator or designee will instruct the subject to

use the NRT as per country label for up to 3 months (+2 weeks) after TQD. The use of medication supportive for smoking abstinence other than NRT will not be allowed.

Any concomitant medication which has a potential impact on the study endpoints, including non-steroidal anti-inflammatory drugs (NSAIDs) including over-the-counter products should be avoided and carefully considered.

Table 1 provides an overview of selected medication and their potential impact on clinical risk endpoints.

To provide additional information and guidance to the Investigator or designee, Appendix 2 provides an overview of drug half-lives for drugs considered to have an impact on some of the study endpoints listed below in Table 1.

In case drugs/short-and long-term vitamins listed in Table 1 are taken by the subject from the enrolment onwards, they will be recorded as concomitant medications (except when salbutamol is used for post-bronchodilator spirometry testing), but this will not be a reason to discontinue the subject from the study.

Table 1 Concomitant Medication and Impact on Clinical Risk Endpoints

Concomitant Medication	HDL-C	WBC	FEV_1	11-DTX-B2	8-epi PGF2α
Vitamins B:	•				
- Thiaminchlorid (Vitamin B1)					
- Nicotinic acid (Vitamin B3)					
- Pyridoxin (Vitamin B6)					
- Cyanocobalamin (Vitamin B12)					
Ascorbic acid (vitamin C)	•				
Tocopherol (vitamin E)					•
Non-steroidal anti-inflammatory drugs (NSAID)		•		•	
Antivirals/antibiotics		•			
Short acting β agonists, long acting β agonists (*e.g.,* salbutamol)*			•		
Aminophylline/theophylline			•		
Systemic broncholytic drugs (*e.g.,* terbutalinsulfat)			•		

Concomitant Medication	HDL-C	WBC	FEV_1	11-DTX-B2	8-epi PGF2α
Tiotropium, anticholinergic bronchodilator			•		
Inhaled Glucocorticosteroids			•		
Antiplatelet agents (*e.g.,* phenprocoumon, apixaban, warfarin)					•
Antidiabetic drugs (thiazolidinediones (Pioglitazone, Rosiglitazone))	•			•	
Beta-blockers (*e.g.,* atenolol, metoprolol)			•		
Angiotensin converting enzyme (ACE) inhibitors (*e.g.,* captopril, enalapril, lisinopril, and ramipril).				•	
Statins / other cholesterol-lowering drugs (*e.g.,* fluvastatin, simvastatin, ezetimibe)	•			•	
Antidepressant (*e.g.,* bupropion)		•			
Estrogen				•	

* Except when salbutamol is used for post-bronchodilator spirometry testing.

7 STUDY PROCEDURES

Personnel performing study assessments must have appropriate and documented training. An overview of all study assessments is shown in the schedule of events (Appendix 1). Site personnel will adhere to the site's standard operating procedures (SOPs) for all activities. Appropriate medical advice will be provided to the subject in case of any medical findings requiring health care.

7.1 Informed Consent and Guidance

Prior to any study assessments being performed, the subject will be asked to provide his/her written consent to participate to the study (ICF) (section 1.3). All assessments must start after the time of ICF signature by the subject for study participation.

In addition to the ICF for study participation, the subject will be asked to provide his/her separate consent for sample bio-banking (section 1.3.2):

- ICF for the additional bio-banking of serum/plasma/urine samples for further measurements of clinical risk endpoints and BoExp.
- ICF for the additional bio-banking of blood and plasma samples for further transcriptomics and lipidomics analysis.

The subject's participation in the study does not depend on his/her consent for bio-banking and will be separated from the consent for study participation. The different consents will be captured in the CRF.

7.2 Information on the Risk of Smoking and Smoking Cessation Advice

Each subject will be given information on the risks of smoking and SC advice at V1, V2 and in case of discontinuation. Information will be given on an individual basis during a face-to-face meeting between the subject and the Investigator or designee, and may additionally be given in a group session. Details of the interviews will be recorded in the source document.

7.3 Smoking Cessation Support

Smoking cessation support, including SC counseling and behavioral support, will be provided at each visit and between visits throughout the study and whenever requested by the subject (section 6.1). General recommendations on SC support are provided in the SC support plan (separate document).

7.4 Clinical Assessments

Any clinically relevant medical condition detected at V1 has to be documented as a concomitant disease. This also applies to clinically relevant findings in laboratory values, vital signs, spirometry and ECGs detected at V1. Any untoward medical occurrence in a subject detected during the study which was not present at V1 must be documented as an AE. Worsening of a pre-existing condition from V1 onwards will also be documented as an AE.

The results of the clinical assessments described in this section will be recorded in the CRF.

7.4.1 Demographic Data

Demographic data (sex, age, race and/or ethnicity) will be recorded.

See Appendix 1 for the timepoints of assessment.

7.4.2 Questions on Smoking History/Habits and Intention to Quit Smoking.

Subjects will be asked the following questions about their smoking history and habits:

5. Have you smoked for at least the past 10 years? (yes/no)
6. How many years have you smoked? (numeric response, 2 digits)
7. On average, how many cigarettes per day have you smoked over the last year? (numeric response, 2 digits)
8. On average, how many cigarettes per day have you smoked since you started smoking? (numeric response, 2 digits)
9. On average, how would you describe your e-cigarette use over the last year? (check one)
    a. Daily.
        i. How much use per day? (numeric response, 2 digits)
    b. Weekly.
        i. How much use per week? (numeric response, 2 digits)
    c. Sporadically. (less than once per week)
    d. Tried e-cigarettes. (between 1 – 10 uses)
    e. Never tried e-cigarettes.

This self-reported CC daily consumption at V1 and V2 will be used to assess eligibility.

Intention to quit smoking within the next 30 days will be assessed at V1 by the means of the Prochaska's questionnaire (section 7.7.1.1).

See Appendix 1 for the timepoints of assessment.

7.4.3 Medical History and Prior 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 started prior to and is still ongoing at V1, or is detected at V1.

Medications will be allowed and carefully monitored during the study by the Investigator or designee. The Investigator or designee is responsible for the medical care including medication of the subjects during their participation in the study. Any decisions regarding the prescribed medications will be made in the best interest of the subject. Any use of concomitant medication must be fully documented in the source document and transcribed into the CRF.

All medication taken 4 weeks prior to V1 (prior medications) and all concomitant medication taken during the study will be documented in the source documentation and recorded in the CRF. Medication which was started prior to V1 and which is still being taken by the subject during the study as well as medication that is initiated after V1 will be considered as a concomitant medication. This applies to both prescription and over-the-counter products (*e.g.,* vitamins). NRT will be recorded as a concomitant medication.

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 start and if applicable, the stop date (day, month and year). Any therapy changes (including changes of regimen) during the study have to be documented. Any concomitant medication that is still being taken by the subject at the EOS will be recorded in the CRF.

7.4.4 Physical Examination

A complete physical examination, including auscultation and palpation will be performed.

See Appendix 1 for the timepoints of assessment.

7.4.5 Body Height, Weight and Waist Circumference

Body weight, height and waist circumference will be measured. The same scale should be used for all assessments.

Appropriate medical advice will be provided to the subject in case any medical findings requiring health care are identified.

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

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

At V1, BMI will be calculated from the height and weight recorded at V1. At the following visits, BMI will be calculated from the height at V1 and body weight recorded at each respective visit.

See Appendix 1 for the timepoints of assessment.

7.4.6 Vital Signs

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

See Appendix 1 for the timepoints of assessment.

7.4.7 Other Clinical Assessments

7.4.7.1 Lung Function Testing

See Appendix 1 for the timepoints of assessment.

All personnel performing lung function 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 lung function testing. All lung function maneuvers will be recorded with the subject in a sitting position throughout the study. 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 or designee.

Spirometry testing

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. Spirometry testing will be managed by a central provider, including the provision of equipment and site manual. The Pulmonary Function Testing (PFT) manual will include information on equipment, procedures, subject instructions and precautions.

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 PFT site manual [20].

All spirometry testing must be performed at least 1 hour after smoking (if applicable).

Pre- and post-bronchodilator spirometry testing

Pre and post- bronchodilator spirometry assessments will be performed. Each assessment requires at least three 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 FEV_1 to FVC at V1 will be used for eligibility criteria to assess spirometry and asthma conditions.

For all the other visits, pre- and post-bronchodilator spirometry will be used to describe the changes in pre- and post-bronchodilator spirometry measurements over the duration of the study. Values for FEV_1, FVC, and FEF 25-75 will be recorded. The ratio FEV_1/FVC will be calculated from the highest acceptable FEV_1 and the highest acceptable FVC respectively. In case, the tests do not meet the acceptability criteria the subject will need to come back within a 5-day window to repeat the tests. More details will be provided in the Pulmonary Function Testing (PFT) manual.

All post-bronchodilator 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.

See Appendix 1 for the timepoints of assessment.

Lung volume measurements using multiple breath helium dilution technique

All lung volumes measurements will be done pre-bronchodilator.

Lung volume measurements will be conducted as part of the lung function tests in selected and specialized centers and the following values will be recorded: VC, FRC, IC and TLC. If required, a second trial can be performed. Between trials, a minimum waiting time of 4 minutes is required. For FRC, the mean value will be used if more than one attempt is performed. TLC is evaluated at FRC plus IC. VC will be the highest from FRC-He if more than one attempt is

performed. Residual volume will be calculated as TLC minus VC. VC is the highest from FRC-He wash in.

The helium dilution technique will be used in accordance with the recommendations of the American Thoracic Society/European Respiratory Society (ATS/ERS) Joint Task Force on the standardization of the measurement of lung volumes [21]. This technique is a closed-circuit system where a spirometer is filled with a mixture of helium and oxygen. The closed-circuit rolling seal spirometer will be filled to a starting volume of six liters with a mixture of containing helium, oxygen and balance room air. Oxygen will be set to 30% so that all test subjects will be comfortable; exact contents will be analyzed. The subject will be asked to seal their lips around the mouthpiece and breathe normally on the closed-circuit while the helium mixes and equilibrates. During this time carbon dioxide will be removed by a chemical absorber and oxygen will be automatically replaced. Once equilibration has occurred, the subject will be asked to perform one or more vital capacity efforts to end the test.

See Appendix 1 for the timepoints of assessment.

7.4.7.2 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 5 minutes in a supine position.

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 and Federici's formula. Every ECG has to be assessed as normal, abnormal – clinically not relevant, or abnormal – clinically relevant. A diagnosis has to be provided on the eCRF for all ECGs assessed as abnormal – clinically relevant. All ECG print-outs will be interpreted by a qualified physician. Any print-outs of ECGs on thermo-sensitive paper must be photocopied and stapled together for inclusion in the source documents and signed by Principal Investigator or designee.

See Appendix 1 for the timepoints of assessment.

7.5 Laboratory Assessments

7.5.1 Assessments in Blood

7.5.1.1 Hematology and Clinical Chemistry

The following parameters will be measured:

Table 2 Hematology and Clinical Chemistry Parameters (Safety)

Hematology	Clinical Chemistry

• Hematocrit
• Hemoglobin
• Mean corpuscular hemoglobin (MCH)
• Mean corpuscular hemoglobin concentration (MCHC)
• Mean corpuscular volume (MCV)
• Platelet count
• Red blood cell (RBC) count
• White blood cell (WBC) count
• Differential WBC count:
  • Neutrophils
  • Basophils
  • Eosinophils
  • Lymphocytes
  • Monocytes
	• Albumin
	• Total protein
	• Alkaline phosphatase (AP)
	• Alanine aminotransferase (ALT)
	• Aspartate aminotransferase (AST)
	• Blood urea nitrogen (BUN)
	• Creatinine
	• Fasting glucose
	• Gamma-glutamyl transferase (GGT)
	• Lactate dehydrogenase (LDH)
	• Potassium
	• Sodium
	• Total and direct bilirubin
	• Total cholesterol
	• Triglycerides

See Appendix 1 for the timepoints of assessment.

7.5.1.2 Serology

A test for hepatitis B surface antigen, hepatitis C virus and human immunodeficiency virus (anti-HIV1/2) will be done at V1.

7.5.2 Assessments in Urine

7.5.2.1 Urine Analysis

The following parameters will be analyzed semi-quantitatively in urine:

- pH
- Bilirubin

- Glucose
- Nitrite
- Red blood cell traces
- Protein
- Specific gravity

See Appendix 1 for the timepoints of assessment.

7.5.2.2 Urine Drug Screen

The urine will be screened for the following drugs or class or drugs: amphetamines, barbiturates, benzodiazepines, cannabinoids, cocaine and opiates.

See Appendix 1 for the timepoints of assessment.

7.5.2.3 Urine Cotinine Tests

In this study, urine cotinine will be analyzed in three different ways. Two different tests will be used at site and a third analysis will be carried out by a central laboratory.

A urine cotinine test will be performed to confirm the subject's smoking status as smoker at Screening and Baseline Visits. The test must detect cotinine with a cotinine threshold of ≥ 200 ng/mL.

Another urine cotinine test in spot urine at site will be used to verify continuous smoking abstinence at each visit from V10 onwards. The cut-off value defined for this smoking abstinence verification is < 100 ng/mL.

Repeat testing for the urine cotinine test in spot urine at site is permitted in case of unclear results.

Subjects who have completed the study before the effective date of the current study protocol (Final Version 7.0) without having ever been tested for cotinine in urine by the urine cotinine test (cut-off < 100 ng/ml) in spot urine at site will not be recorded as subjects with a protocol deviation hereof.

Subjects who have already performed one or several Visits from Visit 10 onwards before the effective date of the current study protocol (Final Version 7.0) without having been tested for cotinine in urine by the urine cotinine test (cut-off < 100 ng/ml) in spot urine at site will not be recorded as subjects with a protocol deviation hereof.

The urine cotinine test used at Screening and at Baseline and the one used at each visit from V10 onwards are two different tests and cannot be exchanged.

See Appendix 1 for the timepoints of assessment.

A third analysis of cotinine in urine will be performed by a central laboratory as a criterion for discontinuation (see section 5.3 and 6.2), i.e. free cotinine concentration (part of the nicotine equivalents) will be determined in 24-hour urine collected at Visit 11. If the concentration of free cotinine is ≥ 50 ng/mL, the subject will be discontinued at the time the results are made available.

7.5.2.4 Urine Pregnancy Testing

All female subjects will perform pregnancy tests. Female subjects with a positive pregnancy test at V1 or V2 will not be enrolled and will be considered as screen failures. In case of any positive urine pregnancy test, the Investigator or designee will inform the subject about the risks associated with smoking during pregnancy.

All pregnancies detected during the study must be reported and handled as described in section 8.5.

See Appendix 1 for the timepoints of assessment.

7.5.3 Assessments in Exhaled Breath

7.5.3.1 Exhaled CO Breath Test

Exhaled CO breath test will serve as a verification of compliance to SA during the study conduct. The cut-off value defined for this smoking abstinence verification is ≤ 10 ppm. During the visits on site, it will be measured with a Micro 4 Smokerlyzer® device or similar.

The CO breath test should be conducted in timely conjunction with the blood sampling for COHb when appropriate.

Repeat testing for the CO breath test is not permitted.

See Appendix 1 for the timepoints of assessment.

7.5.3.2 Alcohol Test

Subjects will have a urine or breath alcohol test at V1.

7.6 Biomarkers of Assessments

7.6.1 Biomarkers of Exposure to HPHCs

For normalization of BoExp to HPHCs, creatinine will also be measured in the 24-hour urine samples.

See Appendix 1 for the timepoints of assessment.

The list of BoExp is provided in Appendix 5.

7.6.1.1 Clinical Risk Endpoints

The list of the clinical risk endpoints to be measured in the study is provided in Appendix 4.

For normalization of clinical risk endpoints measured in urine, creatinine will also be measured in the 24-hour urine samples.

The COHb test should be conducted in timely conjunction with the CO breath (section 7.5.3.1).

The samples will be collected irrespective of the time.

See Appendix 1 for the timepoints of assessment.

7.6.1.2 CYP2A6 activity test

CYP2A6 activity will be measured in plasma using the molar metabolic ratio of *trans*-3'-hydroxycotinine to cotinine [22]. CYP2A6 activity drives the metabolism of nicotine to cotinine and subsequent metabolites.

See Appendix 1 for the timepoints of assessment.

7.6.2 Sample Handling, Storage, and Shipment

All blood and urine samples will be tested at a central laboratory. The urine pregnancy tests and cotinine dipstick tests will be done by personnel at the study sites.

Detailed procedures for collection and handling of samples are described in a separate Investigator Laboratory Manual (ILM). Safety laboratory samples will be destroyed as per the laboratory's standard procedures. All primary and back-up samples for the assessments of clinical risk endpoints and BoExp (except bio-banking samples) of discontinued subjects will be destroyed after all bioanalytical reports have been finalized or the database has been locked, whichever comes last. Personnel at the facility/-ies where samples are stored will be informed in writing by the Sponsor when destruction of the samples will be allowed.

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

7.6.3 Blood Samples

Blood samples will be collected by qualified and trained site personnel. Subjects should be in a seated position during blood collection. The maximal total volume of blood drawn for each subject will be around 375 mL, which includes about 65 mL for safety and repeated analysis, about 50 mL for long-term storage of the bio-banking samples for further analysis of BoExp and clinical risk endpoints (only if additional consent is given) and 40 mL for long-term storage bio-banking samples for further analysis of transcriptomics and lipidomics (only if additional consent is given) (section 7.6.5).

The blood sampling for transcriptomics & lipidomics, and the data related to these samples will be anonymized. Anonymized data and samples are initially single or double coded where the link between the subjects' identifiers and the unique code(s) is subsequently deleted.

7.6.4 Urine Samples

7.6.4.1 Spot Urine Samples

Spot urine samples will be used for the urine drug screen, urine cotinine tests, urine pregnancy test and safety urine analysis.

7.6.4.2 24-hour Urine Collection

Twenty four-hour urine fraction will be collected.

The day before the visit, subjects will start collection of 24-hour urine at home. Subjects will discard their first void in the morning. The collection period will start immediately after. After 24-hour ± 1 hour of urine collection, in the morning of the visit, subjects will empty their bladder again and this urine will be used as the final portion of the 24-hour urine sample.

During the collection period, all urine passed must be collected and put into the sampling container. No urine must be passed into the toilet. The start and the end time of urine collection will be recorded by the subject in a form and checked by the site staff. The volume of 24-hour urine will be measured by the site staff upon collection of urine containers from the subjects.

For assessment of urine BoExp, creatinine, 8-epi-$PGF_{2\alpha}$ and 11-TBX-B2, albumin and sample for bio-banking (if consent received), aliquots from the 24-hour urine collection will be taken. In the table of assessments, for the 24-hour urine collection, the dot corresponds to the day on which the 24-hour urine collection period ends.

See Appendix 1 for the timepoints of assessment.

7.6.5 Bio-banking for Long Term Storage of Blood and Urine

If a subject gives consent for sample bio-banking for further analysis of BoExp and clinical risk endpoints, additional samples of urine (from the 24-hour urine collection) and serum/plasma (50 mL of blood in total) will be collected as follows:

- From the 24-hour urine collections 5 aliquots of 10 mL urine for each visit.
- Serum/plasma: at each visit 2 tubes with about 5 mL of blood each will be filled and centrifuged: from one tube, two aliquots of 1 mL plasma and from the second tube 2 aliquots of 1 mL of serum will be collected and stored.

If a subject gives consent for sample bio-banking of whole blood/plasma for further transcriptomics & plasma for lipidomics analysis, an additional 9 mL of blood will be collected for each assessment: 5 mL for transcriptomics will be aliquoted in 2 tubes (2.5 mL of blood/tube); for lipidomics, the 4 mL of blood will be centrifuged and the plasma aliquoted in 2 tubes of 500 µl each.

The samples intended for sample bio-banking will be kept frozen, separated from the other samples collected, and will be shipped to a central storage facility according to the ILM. After the final CSR is signed, samples of plasma/serum/blood will be stored for a maximal period of 5 years and samples of urine will be stored for a maximal period of 2 years. The blood/plasma bio-banking for transcriptomics and lipidomics will be stored for a maximum period of 5 years.

If a subject withdraws his/her consent for sample bio-banking the facility at which the samples are stored will follow their procedures for destruction of banked samples as stated in section 1.3.2.

See Appendix 1 for the timepoints of assessment.

7.7 Other Study Procedures

7.7.1 Questionnaires

The subject questionnaires and the cough-VAS used in this study will be entered by the subject directly in a subject reported outcomes (SRO) device or on paper copy. All subject reported outcome data as well as instructions will be provided in the subject's local language. The questionnaires and the cough-VAS will be reviewed for completeness by the study site staff and subjects will be requested to complete any missing information.

Symptoms or worsening of symptoms documented on any of the questionnaires or on the VAS do not need to be documented as additional AEs because the questionnaires and the cough-VAS will be analyzed as part of the final report. However, it is at the discretion of the Investigator or designee 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 the study site staff, using open, non-directive questions (section 8.2).

See Appendix 1 for the timepoints of assessment.

7.7.1.1 Prochaska 'Stage of Change' Questionnaire: Intention to Quit Smoking

The Prochaska's 'Stage of Change' questionnaire will be used to assess the smokers' mental state for the intention to quit (Appendix 2 for staging algorithm; [23, 24].There are 5 stages of change: 1. *Precontemplation*, 2. *Contemplation*. 3. *Preparation*. 4. *Action.* 5. *Maintenance*. In the *Precontemplation* stage, the individual does not recognize smoking as a problem.

In the *Contemplation* stage, the individual is gathering information about smoking, such as contacting a health care provider or a tobacco quit line for information on the effects of smoking or smoking cessation consequences. During this stage, the stress and inconvenience of quitting smoking is greater than the immediate and possible long-term health effects of continuing smoking. In the *Preparation* stage, intention and behavior begin to come together and the subject is preparing to enter into action in the next 30 days. It is necessary for the subject to recognize the benefits of not smoking, before a subject can enter the *Action* stage and as a result, changes his/her smoking behavior. After six months of not smoking, the individual reaches the *Maintenance* stage when different skills may be needed to prevent relapse from smoking.

All subjects who are planning to quit smoking within the next 30 days will be eligible for the study regardless of having one day of quit attempt over the past year.

See Appendix 1 for the timepoints of assessment.

7.7.1.2 Fagerström Test for Nicotine Dependence (revised version)

Potential nicotine dependence will be assessed via a questionnaire using the Fagerström test for nicotine dependence (FTND) in its revised version [25].

The questionnaire consists of six questions which will be answered by the subject himself/herself. The scores obtained on the test allow the classification of nicotine dependence in three different levels: mild (0-3 points), moderate (4-6 points), and severe (7-10 points).

See Appendix 1 for the timepoints of assessment.

7.7.1.3 Assessment of Cough-VAS

Subjects will be asked to assess the respiratory symptom 'cough' on a VAS, on three Likert scales, and with an open question. Assessment of cough will be conducted irrespective of the time.

Subjects will be asked if they have experienced a regular need to cough, *e.g.*, whether they have coughed several times in the previous 24 hours prior to assessment. If the answer is 'yes', subjects will be asked to complete a VAS, 3 Likert scales and to answer the open question.

On the VAS, subjects will assess how bothersome their cough was during the previous 24 hours. The VAS ranges from 'not bothering me at all' to 'extremely bothersome.'

Furthermore, subjects will assess the intensity and frequency of cough and the amount of sputum production during the previous 24 hours on Likert scales.

The intensity of cough will be assessed on a 5-point Likert scale ranging from 1 to 5, with 1 = very mild – 2 = mild – 3 = moderate – 4 = severe – 5 = very severe.

The frequency of cough will be assessed on a 5-point Likert scale ranging from 1 to 5, with 1 = rarely – 2 = sometimes – 3 = fairly often – 4 = often – 5 = almost always.

The amount of sputum production will be assessed on a 4-point Likert scale ranging from 0 to 3, with 0 = no sputum – 1 = a moderate amount of sputum – 2 = a larger amount of sputum – 3 = a very large amount of sputum.

Finally, subjects will be asked to share any other important observations with the staff about their coughing.

See Appendix 1 for the timepoints of assessment.

7.7.1.4 Socio-Economic Status

The SES information will be collected in the following countries: US, UK, Poland, Germany and Japan.

As part of the characterization of the study population it is important to measure variables that have been shown to be related to nicotine dependence and tobacco product reinforcing value. Based on prior tobacco research these factors include age, gender, ethnicity, tobacco use history, educational as well as socio-economic status.

SES information is recorded in similar manner in the clinical program, in behavioral research and will be eventually assessed in post-marketing studies. In order to evaluate factors affecting the success for a 12-month SC, the socio-economic status constitutes an important demographic parameter. Socio-economic status data will be reported across the randomized

clinical studies and will be collected in observational pre- and post-marketing studies. At V1 the subjects will be informed in details about the exams and evaluations planned during the study, and similarly notified about the SES assessments which will be done at V2. After having provided informed consent at V1, the subjects will be enrolled in the study.

Subjects will be asked a series of questions related to their education, occupational status, size and annual income of their household. These data will be used to create a measure for SES that categorizes subjects into low, moderate and high SES [26]. If the subject does not want to answer the questionnaire, he/she will not be withdrawn from the study.

See Appendix 1 for the timepoints of assessment.

7.7.1.5 Lifestyle Assessment

Subjects will be asked questions to capture baseline covariates such as diet, alcohol intake, sleep deficit, exercise and exposure to passive smoking.

See Appendix 1 for the timepoints of assessment.

8 ADVERSE EVENTS

8.1 Definitions

8.1.1 Adverse Events

Since no investigational products will be used in this study, any health-related event occurring during the study will be reported.

For the purpose of this study an AE is defined as any untoward medical occurrence that may present itself during the conduct of a study and which may or may not have a causal relationship with the study procedures. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom or disease.

8.1.2 Serious Adverse Events

A SAE is defined as, but is not limited to, any untoward medical occurrence 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, an SAE may jeopardize the subject or the subject may require medical or surgical intervention to prevent one of the outcomes listed in the above definitions.

Any pre-planned hospitalizations that are known at the time of signing the ICF will not be recorded as an SAE; however they will be recorded as AE only. Any AE that occurs during this pre-planned hospitalization will be considered according to the above definitions.

8.2 Assessment of Adverse Events

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

8.2.1 Collection of Information

Adverse event information will be collected from the time of signature of the ICF onwards until EOS either by the Investigator or designee via spontaneous reporting or by the use of consistent, open, non-directive questions from study site staff (*e.g.*, "Have you had any health problems since the previous visit/How are you feeling since you were last asked?"). The main source for AE collection will be face-to-face interview(s) with the subject.

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

For each AE, the intensity will be graded on a 3-point intensity scale (mild, moderate, severe) using the definitions provided in section 8.2.3.

Any exacerbation/worsening or increased frequency of an AE or pre-existing condition shall be evaluated and recorded.

Correct medical terminology/concepts are preferred when recording AE terms, and abbreviations must be avoided. Wherever possible, a diagnosis is to be used to describe an AE as a diagnosed medical condition rather than individual signs and symptoms (*e.g.*, record 'pneumonia' rather than 'fever', 'cough', 'pulmonary infiltrate' or 'septicemia' rather than 'fever' and 'hypotension' following blood sample).

Any AE that meets the seriousness criteria must be recorded both on the AE report form of the CRF and on a separate SAE report form (section 8.2.3).

8.2.2 Period of Collection

From the time of signature of the ICF onwards until EOS, all AEs (includes SAEs) will be collected by the study site staff as described below.

8.2.2.1 Screening Period

All existing health conditions identified during the Screening Period will be recorded as concomitant disease and the subject's eligibility for Baseline to the study will be reviewed. Any AEs which occur during the Screening Period will be captured by the study site staff and assessed by the Investigator or designee in order to establish relationship or relatedness in respect to study procedures. All collected AEs will be reported in the CSR and will be in accordance with the respective regulatory guidelines.

8.2.2.2 From Enrolment until the End of the Study

Any new, clinically relevant, abnormal finding detected during the study or worsening of a pre-existing condition/concomitant disease will be documented as an AE and/or SAE.

All ongoing AEs at the EOS will not be followed-up by the Investigator or designee. At the discretion of the Investigator, the subject will be referred to his/her General Practitioner for follow up on ongoing AEs (section 8.3).

8.2.3 Intensity of Adverse Event

For each AE, the intensity will be evaluated by the Investigator or designee on a 3-point intensity scale (mild, moderate, severe) using the following definitions:

Mild: The AE is easily tolerated and does not interfere with daily activity.

Moderate: The AE interferes with daily activity, but the subject is still able to function.

Severe: The AE is incapacitating and requires medical intervention.

Change in severity (intensity) needs to be documented.

8.2.4 Relationship to Study Procedures

In general, all AEs and/or SAEs will be assessed by the Investigator or designee as either 'related' or 'not.

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.

8.3 Reporting of Serious Adverse Events

Any SAEs reported or observed during the study after signature of the ICF until EOS, whether or not attributable to any medication or to any study procedures must be reported by the Investigator or other study site staff **within 24 hours after first awareness by any party involved in the study** to United BioSource Corporation (UBC) Safety and to the Sponsor.

An SAE report form must be faxed or e-mailed as an attachment to:

UBC Safety:	**Fax number:**	[redacted]
	E-mail:	[redacted]
	Address:	United BioSource Corporation [redacted].
Sponsor Contact: [redacted], MD, Medical Safety Officer	**Phone:**	[redacted]
	E-mail 1:	[redacted]
	E-mail 2:	[redacted]
	Address:	Philip Morris Products S.A. R&D Innovation Cube Quai Jeanrenaud 5 2000 Neuchâtel, Switzerland

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

Any SAE will be reported to the competent authorities by the Sponsor as per local requirements.

Any additional/follow-up information that becomes available after the initial SAE report form has been completed will be forwarded to UBC Safety and the Sponsor within 24 hours after first awareness by any person at the site using a follow-up to the existing SAE report form.

The follow-up SAE report form must include the minimum information required as described in the safety management plan for form completion and only modified or new information needs to be specified. Information provided in the follow-up SAE report form supersedes any information that was initially reported.

All SAEs will be followed up by the Investigator or designee and/or UBC Safety until their resolution or until the Investigator or designee considers the event to be stabilized (*i.e.*, no worsening of condition), or until an acceptable explanation has been found (*e.g.*, a chronic condition).

The SAE report form to be used in this study is provided as a separate document and included in the study master file. All SAEs will be recorded, in addition to the SAE report form.

8.4 Reporting of Other Events Critical to Safety Evaluations

8.4.1 Abnormal Results of Laboratory Tests

Any clinical safety laboratory test result that is outside of the normal reference range will be reviewed by the Investigator or designee and assessed for clinical relevance. If an abnormal laboratory result is detected after Screening and considered clinically relevant (see below), this should be recorded as an AE.

The grading scheme shown in Appendix 6 [27] will be used by the Investigator or designee to assess abnormal laboratory AEs as follows:

- All Grade 1 abnormal laboratory values will be evaluated by the Investigator or designee with respect to baseline value and clinical relevance. If considered to be clinically relevant the Investigator or designee must report it as an AE. All Grade 2 and higher abnormal laboratory values must be reported as, or linked to, an AE/concomitant disease.
- If a subject has Grade 2 and higher abnormal laboratory values at V1 it is at the discretion of the Investigator or designee to enroll the subject or not. This decision must be documented in the source document and captured in the CRF. A grade 2 and higher laboratory abnormal value at V1 must be recorded as concomitant disease.
- If there is any worsening in grade from Grade 2 and above during the study the Investigator or designee must report this worsening as an AE.
- Where there is no grading available, the abnormal laboratory value will be evaluated by the Investigator or designee and assessed for clinical relevance. If considered to be clinically relevant, the Investigator will report it as an AE.
- Any other abnormal clinical laboratory result (including those that are not part of the core safety assessments) can, at the discretion of the Investigator or designee, be reviewed and assessed. Even if they do not meet the criteria of the CTCAE grading scheme (please see above), the Investigator or designee may consider them to be of clinical relevance and, if they are, must report them as AEs.
- In general, laboratory values will be recorded as ‘increased <lab parameter>’ or ‘decreased <lab parameter>’ to ensure consistency of recording/coding.

All other information (*e.g.*, intensity, seriousness, outcome) will be assessed as for other AEs.

8.5 Reporting and Follow-Up of Pregnancies

All subjects who are diagnosed as pregnant after enrolment (V2) will be discontinued from the study. Smoking cessation counseling will be provided to those subjects who are still abstinent from smoking and will be referred to the respective health care facility/health care provider for further support. In case a subject has relapsed to smoking and is diagnosed as pregnant, advice on the risk of smoking and smoking cessation advice will be provided and subjects will be referred to the respective health care facility/health care provider for further support.

Pregnancy Forms will be filled but no follow-up on pregnancy outcome will be performed.

The Investigator or designee is responsible for informing the IRB/IEC of any pregnancy that occurs during the study and its outcome, according to local regulations.

8.6 Adverse Events Leading to Discontinuation

Subjects who are discontinued from the study because of an AE, will undergo the early termination procedures as described in section 9.67 as soon as possible. The Investigator or designee and/or UBC Safety will follow up these AEs until they have been resolved, stabilized (*i.e.*, no worsening of condition), or until an acceptable explanation has been found.

9 STUDY ACTIVITIES

A detailed schedule of assessments can be found in Appendix 1.

In general, if no start time for the procedures is provided, the procedure can be performed at any time during the day, but should be done at the similar or comparable time for each visit.

For the details on the procedures, see the related sections.

9.1 Screening Visit (V1)

The Screening (V1) will be performed within 6 weeks (1 to 42 days) prior to V2. First, the ICF along with study information should be given to the subject. When/if the ICF is signed, dated and timed, the other screening procedures can be performed in the order deemed most practical. While it is recommended to complete as many screening procedures as possible in one day, it is permissible to complete those over more than one day. Smoking is allowed during screening.

Table 3 shows the procedures for V1. The sequence of assessments/events is given just for illustrative purposes. It will be at the discretion of the site after signature of the ICF.

Table 3 Time Schedule – Screening Visit (V1)

Time	Blood Sample	Procedures	Additional Information
Start of Procedure		**Screening**	
Before any study procedure		Informed consent for study participation and additional consents for bio-banking (if applicable)	Lung volumes will be part of the main ICF in selected centers only.
During the visit	√	Clinical laboratory parameters (hematology, clinical chemistry)	
During the visit	√	Serology (HIV, hepatitis B and C)	
During the visit		Information on the risk of smoking and SC advice	
During the visit		Demographic data collected	
During the visit		Medical history/concomitant disease, prior and concomitant medication	

Time	Blood Sample	Procedures	Additional Information
Start of Procedure		**Screening**	
During the visit		Questions on smoking history/habits	
		Readiness to comply with study procedure including to remain continuously abstinent from smoking for 52 weeks	
During the visit		Prochaska questionnaire (Intent to quit)	
During the visit		Lifestyle questions	
At any time		ECG	At least 5 minutes in supine position prior to recording.
During the visit		Vital signs (blood pressure, pulse rate, respiratory rate)	At least 5 minutes in supine position
During the visit		Urine analysis	
During the visit		Urine pregnancy test (all females)	
During the visit		Physical examination, height and weight.	
During the visit		Urine drug screen	
During the visit		Urine cotinine test	Cut-off ≥ 200 ng/ml
During the visit		Alcohol urine or breath test	
During the visit		Spirometry pre- and post-bronchodilatator (recording of FEV_1, FVC, FEV_1/FVC and FEF 25-75 only)	Has to be done at least 1 hour after smoking At rest for at least 15 minutes prior to pulmonary function testing. In sitting position. All post-bronchodilator spirometry testing will be performed 15-30 minutes post administration of salbutamol

Time	Blood Sample	Procedures	Additional Information
Start of Procedure		**Screening**	
During the visit		AE/SAE recording	If the Screening Visit is performed on two separate days, the AE/SAE questions will be asked again
During the visit		Inclusion/exclusion criteria	
At discharge		Instructions and distribution of urine containers	A paper form will be distributed to the subject for recording the start and the end time of urine collection.

Abbreviations: AE = Adverse event; BoExp: Biomarkers of exposure; BMI = Body mass index; ECG = Electrocardiogram; FEF = Forced expiratory flow; FEV_1= Force expiratory volume in 1 second; FVC = Forced vital capacity; HIV = Human immunodeficiency virus; SAE = Serious adverse event.

9.2 Baseline Visit (V2)

Table 4 shows the assessments that will be performed on the day of V2. Assessments must be done after enrolment, unless specified.

Table 4 Time Schedule – Baseline Day (V2)

Time	Blood Sample	Procedures	Additional Information
Start of Procedure			
During the visit		End of 24-hour urine sample collection V2 container	Prior to enrolment, staff should check that the subject brought his/her urine containers filled with urine The start of urine collection is at subject's home the day before V2 and the end is in the morning of V2. The urine is collected for 24 hours ±1 hour.

Time	Blood Sample	Procedures	Additional Information
Start of Procedure			
During the visit		Questions about smoking history/habits	Prior to enrolment
During the visit		Inclusion criteria n°4 and 6	Prior to enrolment
During the visit		Exclusion criteria n° 5 and 6	Prior to enrolment
During the visit		Urine cotinine test	Prior to enrolment
			Cut-off ≥ 200 ng/ml
During the visit		Urine pregnancy test (all females)	Prior to enrolment
During the visit		Enrolment	After all inclusion and exclusion criteria have been satisfactorily met and subject has provided filled urine containers
		Urine sampling to be taken from the 24-hour urine V2 container	Samples will be collected for some clinical risk endpoints and the full list of BoExp.
			Must be done after enrolment
During the visit		Urine analysis	Must be done after enrolment
During the visit	√	Clinical laboratory parameters (hematology, clinical chemistry)	After at least 10 hours of fasting
			Must be done after enrolment
During the visit	√	CVD Clinical risk endpoints	After at least 10 hours of fasting
			Must be done after enrolment
During the visit	√	Serum/plasma for bio-banking for BoExp/clinical risk endpoints and blood/plasma transcriptomics & lipidomics	If additional consent is signed
			After at least 10 hours of fasting

Time	Blood Sample	Procedures	Additional Information
Start of Procedure			
			Must be done after enrolment
During the visit	√	Nicotine in plasma	Must be done after enrolment
During the visit	√	CYP2A6 activity (including cotinine) in plasma	Must be done after enrolment
During the visit	√	COHb in blood	Has to be done in conjunction with CO breath test. Irrespective of CC use. Must be done after enrolment
During the visit		CO breath test	Irrespective of CC use. Has to be done in conjunction with COHb blood sampling.
During the visit		AE/SAE recording, concomitant medications	At any time of the day
During the visit		Vital signs (blood pressure, pulse rate, respiratory rate)	At least 5 minutes in supine position Must be done after enrolment
During the visit		Physical examination, weight and waist circumference	Must be done after enrolment
Prior to smoking		Lung function: spirometry pre-bronchodilator, then lung volumes (in selected centers only) followed by spirometry post-bronchodilator	Has to be done at least after 1 hour without smoking Subject at rest for at least 15 minutes prior to lung function testing. In sitting position. All post-bronchodilator spirometry testing will be performed 15-30 minutes

Time	Blood Sample	Procedures	Additional Information
Start of Procedure			
			post administration of salbutamol Needs to be done in the morning prior to 12:00 PM but after enrolment
During the visit		Cough-VAS and SES questionnaires	Must be done after enrolment
During the visit		Lifestyle questions	Must be done after enrolment
During the visit		FTND questionnaire	Must be done after enrolment
Before check-out		Information on the risk of smoking and SC advice.	Must be done after enrolment
During the visit		Smoking cessation support	At this visit, the Investigator or his/her designee will provide recommendation to the subject for NRT, if NRT use is needed. Must be done after enrolment
Before check-out		Define individual TQD	Must be done after enrolment

Abbreviations: AE = Adverse event; BoExp = Biomarkers of exposure; CVD: Cardiovascular diseases; CO: Carbon monoxide; COHb = Carboxyhemoglobin; CYP = Cytochrome P450; FTND = Fagerström test for nicotine dependence; NRT = Nicotine replacement therapy; SES= Socio-economic situation; SAE = Serious adverse event; VAS = Visual analog scale.

9.3 Visit 3 (V3)

The subject will be asked to come to the clinic within 24 to 48 hours after the TQD defined at V2. Table 5 shows the assessments that will be performed on V3.

Table 5 Time Schedule – Target Quit Date (V3)

Time	Procedures	Additional Information
Start of Procedure		
During the visit	AE/SAE recording	
During the visit	SC support	At this visit, the Investigator or his/her designee will provide recommendation to the subject for NRT, if NRT use is needed.
During the visit	Concomitant medications and record preferred NRT (if applicable)	

Abbreviations: AE = Adverse event; SAE = Serious adverse event; NRT = Nicotine replacement therapy.

9.4 Actual Quit Date

No visit on site will take place at the AQD. However, the AQD will serve as a starting point (date) to establish dates of all subsequent visits.

9.5 Smoking Abstinence Period (V4 to V17)

Table 6 describes the assessments on V4, V5, V6, V7, V9, V10, V12, V13, V14, V15 and V16 and Table 7 the assessments of V8, V11 and V17. A time window of ± 8 days is allowed for these visits, with the exception of V4 and V5: ± 3 days. The collection of 24-hour urine will start at home. The period from check-out of V2 to AQD has been established in order to identify subjects more likely to remain continuously abstinent from smoking for the whole duration of the study.

Table 6 Time Schedule – Visits V4, V5, V6, V7, V9, V10, V12, V13, V14, V15 and V16

Time	Blood Sample	Procedures	Additional Information
Start of Procedure			
During the visit		AE/SAE recording	

During the visit	Urine pregnancy test (all females).	
During the visit	Urine cotinine test	From Visit 10 onwards. The cut-off value defined for this verification of continuous abstinence from smoking is < 100 ng/mL.
During the visit	SC support and self-reporting by the subject on continuous smoking abstinence.	
During the visit	CO breath test	
During the visit	Vital signs (blood pressure, pulse rate, respiratory rate).	At least 5 minutes in supine position
During the visit	Concomitant medication	

Abbreviations: AE = Adverse event; CO = Carbon monoxide; SAE = Serious adverse event.

Table 7 Time Schedule – Visits V8, V11, and V17

Time	Blood Sample	Procedures	Additional Information
Start of Procedure			
During the visit		Urine cotinine test	From Visit 10 onwards. The cut-off value defined for this verification of continuous abstinence from smoking is < 100 ng/mL.
During the visit		End of 24-hour urine sample collection	The urine is collected for 24 hours ±1 hour. The start of urine collection is at subject's home the day before the visit and the end is in the morning of the visit.
During the visit		Urine sampling to be taken from the 24-hour urine visit container	At V8, samples are collected for some clinical

Time	Blood Sample	Procedures	Additional Information
Start of Procedure			
			risk endpoints and the full list of BoExp. At V11 and V17, samples are collected for some clinical risk endpoints and the reduced list of BoExp.
During the visit		Urine sample to be taken for bio-banking of BoExp and CVD clinical risk endpoints	If additional consent is signed (for bio-banking).
During the visit	√	CVD Clinical risk endpoints	After at least 10 hours of fasting
During the visit	√	Bio-banking for BoExp/clinical risk endpoints and transcriptomics & lipidomics	If additional consent is signed. After at least 10 hours of fasting
During the visit	√	Clinical laboratory parameters (hematology and clinical chemistry)	After at least 10 hours of fasting
During the visit	√	CYP2A6 activity (including cotinine) in plasma	
During the visit	√	Nicotine in plasma	
During the visit	√	COHb in blood	Has to be done in conjunction with CO breath test.
During the visit		CO breath test	Has to be done in conjunction with COHb blood sampling.
During the visit		AE/SAE recording	
During the visit		Urine pregnancy test (all females).	
During the visit		ECG	V11 and V17 only. At least 5 minutes in supine position prior to recording.

Time	Blood Sample	Procedures	Additional Information
Start of Procedure			
During the visit		SC support and self-reporting by the subject on continuous smoking abstinence.	
During the visit		Physical examination, weight and waist circumference.	
During the visit		Urine analysis.	
In the morning		Lung function: spirometry pre-bronchodilator, then lung volumes (in selected centers only) followed by spirometry post-bronchodilator	Subject at rest for at least 15 minutes prior to lung function testing. In sitting position. All post-bronchodilator spirometry testing will be performed 15-30 minutes post administration of salbutamol Needs to be done in the morning prior to 12:00 PM.
During the visit		Vital signs (blood pressure, pulse rate, respiratory rate)	At least 5 minutes in supine position
During the visit		Cough-VAS questionnaire and lifestyle questions	
During the visit		Concomitant medication	

Abbreviations: AE = Adverse event; BoExp = Biomarkers of exposure; CO = Carbon monoxide; COHb = Carboxyhemoglobin; CVD = Cardiovascular disease; CYP= Cytochrome P450; ECG = Electrocardiogram; SAE = Serious adverse event; VAS = Visual analog scale.

9.6 Safety Follow-Up Period and Phone Contact (V18)

A subject who has completed V17, or a subject who has been discontinued from the study prematurely (early termination), will enter a 28-days Safety Follow-Up Period during which spontaneously reported new AEs/SAEs will be recorded, and the active follow-up of ongoing AEs/SAEs will be done by the site. All AEs will be followed-up until resolved, stabilized (i.e.,

no worsening of the event), or a plausible explanation for the event has been found until the end of the Safety Follow-Up Period.

At the end of the Safety Follow-Up Period in Week 56 (V18 (±3 days)), the investigator will attempt to contact only the subject who has previously completed V17 by phone to check if all AEs/SAEs potentially occurring during the Safety Follow-Up period are fully reported and for self-reporting by the subject on continuous smoking abstinence. At the end of the Safety Follow-Up Period, all ongoing AEs will be documented as "ongoing" and will not be followed-up by the Investigator. At the discretion of the Investigator, the subject will be referred to his/her General Practitioner for follow up on ongoing AEs.

If the investigator can reach the subject who has previously completed V17 by phone at the end of the Safety Follow-Up Period in Week 56, the date of this phone call with the subject will be recorded as the date of the end of the study of the subject.

If the investigator cannot reach the subject who has previously completed V17 by phone at the end of the Safety Follow-Up Period in Week 56 after a reasonable number of attempts, the date of the last contact (e.g., last visit of the subject, last phone call with the subject) will be recorded as the date of the end of the study of the subject.

Table 8 Time Schedule – Visit V18

Time	Blood Sample	Procedures	Additional Information
Start of Procedure			
During the phone contact		Self-reporting by the subject on continuous smoking abstinence	
During the phone contact		AE/SAE recording	

9.7 Early Termination Procedures

If a subject is discontinued from the study prematurely, he/she will be advised to perform the following assessments listed in Table 9 below.

Table 9 Time Schedule – Early Termination Procedures

Time	Blood Sample	Procedures	Additional Information
Start of Procedure			
During the visit		AE/SAE recording	
During the visit		Urine pregnancy test (all females)	
During the visit		Urine analysis	
During the visit	√	Clinical laboratory parameters (hematology and clinical chemistry)	
During the visit		Information on the risk of smoking and SC advice	
During the visit		ECG	At least 5 minutes in supine position prior to recording.
During the visit		Physical examination	
In the morning		Lung function: spirometry pre-bronchodilator, then lung volumes (in selected centers only) followed by spirometry post-bronchodilator	Subject at rest for at least 15 minutes prior to lung function testing. In sitting position. All post-bronchodilator spirometry testing will be performed 15-30 minutes post administration of salbutamol Needs to be done in the morning prior to 12:00 PM.
During the visit		Vital signs (blood pressure, pulse rate, respiratory rate)	At least 5 minutes in supine position

Abbreviations: AE = Adverse event; SAE = Serious adverse event.

10 QUALITY CONTROL AND QUALITY ASSURANCE

10.1 Monitoring

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

The Investigator or designee 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 or designee shall access medical records for the Monitor in order that entries in the CRFs may be verified. The Investigator or designee, as part of his/her responsibilities, is expected to ensure that the study adheres to GCP requirements.

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

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

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/or 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, or a designated member of the Investigator's staff, must be available during the monitoring visit to review the data and resolve any queries and to allow direct access to the subject's records for source data verification.

10.2 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 to the relevant systems and other study-specific procedures. The activities of this meeting will be described in the monitoring plan.

Further to the Investigator meeting, the Investigator or designee 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. The Investigator or designee will maintain a record of all individuals involved in the study.

10.3 Audits and Inspections

Good Clinical Practice regulations require 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 and any applicable regulatory requirements. The Investigator or designee will contact the Sponsor or the authorized representative immediately if contacted by a regulatory agency about an inspection at their site.

The Investigator and study staff are 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 or designee understands and agrees to provide access to the necessary documentation and files.

11 DATA MANAGEMENT ACTIVITIES

All data management activities will be described in details in the Data Management Plan (DMP) and documents specified therein. The electronic systems used, CRF and SRO, to collect subject data will be FDA 21 CFR Part 11 compliant.

11.1 Data Capture

11.1.1 Case Report Forms and Study Records

With the exception of the SRO data, all results from the clinical assessments will be recorded in the source data file by the Investigator or his authorized designee(s), and then captured in the CRFs at the study site. 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 transferring the data to the CRF according to the CRF Completion Guidelines.

The Investigator or designee has the ultimate responsibility for the collection and reporting of all data related to the clinical study and ensuring that the data are accurate, authentic/original, legible, timely (contemporaneous), enduring and available when required. The CRF must be signed by the Investigator or designee to attest that the data contained in the CRF are true and accurate. Any corrections made to source documents and/or CRFs must be 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 data will be verified against the source documents at the study site by the Monitor. Instances of missing or unclear data will be discussed with the Investigator or designee for resolution. All SRO questionnaires will be provided to the subject in his/her local language.

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 study endpoints.

All protocol deviations will be entered into 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 but will not be formally reconciled with the CRF database (*e.g.*, their description or occurrence date). 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. 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 enrolled including screening failures and AE during the study (from the time of informed consent to the end of the study of the subject) will be captured in the source documents and all AEs will be entered in the study database (CRF).

All data collected during the study are declared property of the Sponsor, irrespective of the location of the database and the data management CRO.

Additional details are covered in the DMP.

12 PLANNED STATISTICAL METHODS

12.1 General Considerations

Full details of the statistical analysis will be given in a 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.

The data from this study could be used for the purpose of providing data for the design and interpretation of assessment studies of PMI candidate modified risk tobacco products.

12.1.1 Stratification Criteria

For the analysis of the study endpoints, the following stratification criteria will be used:

1. Sex (male; female)
2. Region (US, EU, JP)

12.1.2 Definitions for Statistical Data Analysis

Baseline:

Unless specified, baseline is defined as the last available time point prior to TQD.

12.1.3 Descriptive Statistics

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

In general, summary statistics will be stratified by region and sex at Baseline.

Descriptive statistics (number of subjects [n], number and percent of subjects with missing data, the arithmetic mean, arithmetic standard deviation (SD), median, first and third quartiles, minimum and maximum for continuous data, including geometric mean and coefficient of variation (CV) for data analyzed in the log scale; frequency counts and percentages for categorical data) will be presented overall and at each time point, where applicable.

Apart from FEV_1, WBC, HbA1c, LDL-C, Apo A1, Apo B and HDL-C, for other clinical risk endpoints and BoExp, the geometric mean and CV will be presented in addition to the (arithmetic) mean and SD. Absolute and percent change from Baseline will be summarized for endpoints analyzed in the real and logarithmic scale, respectively.

Descriptive statistics of endpoint parameters will be presented at each assessment time point.

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

Descriptive summaries will be provided for the evaluable data with no imputation.

Missing values for the endpoints analyzed via the mixed model method will not be directly imputed as they are handled within the analysis itself.

Values below the lower limit of quantification (LLOQ) will be imputed using 0.5 x LLOQ. For values above the upper limit of quantification (ULOQ), the ULOQ will be used for calculation and reporting in summary tables. 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 (if no value below LLOQ is present) and maximum (if no value above ULOQ are present) of the observed values.

For questionnaire data, total scores and domain or subscale scores may use a certain degree of imputation by averaging across individual item scores.

Further details will be provided in the SAP.

12.1.5 Significance Level for Inferential Analysis

This study has no formal pre-specified hypotheses associated with the study objectives. However, 95% CI will accompany all effect estimates.

12.2 Determination of Sample Size and Power Consideration

Enrolment is planned for approximately 950 adults with at least 2 weeks of abstinence after the AQD. Unsuccessful quitters will be discontinued from the study. Subjects who discontinued the study after V5 will not be replaced.

Since no formal hypotheses have been formulated for the objectives, sample size calculations are based on precision of effect estimates for FEV_1. Other endpoints are assumed to exhibit a larger effect size than FEV_1 and thus would result in smaller sample size estimates. The sample size of this study is based on our current understanding of the effect and variability of SC from the results of the Lung Health Study [1] in the subject intending to quit smoking at Baseline, since no data on the attrition rate at 12 months after 2 weeks of smoking cessation are available. In particular, approximately 190 subjects are needed to estimate the mean increase from Baseline of 1.98 [% pred.] [1] in FEV_1[4] at V17, with a 90% probability of obtaining a margin

[4] Sample size calculations are driven by FEV_1. Other endpoints are assumed to exhibit a larger effect size than FEV_1 and thus would result in smaller sample size estimates.

of error (95% CI) of at most ±1 [% pred.]. The anticipated SD of the change from Baseline of 6.4 [% pred.] was estimated using the results of the Lung Health Study [1] and includes an inflation of 10% in order to account for additional sources of variability, including the multi-national nature of the study. As the analysis will only be conducted in successful quitters, this sample size was increased to 950, to account for a predicted continuous abstinence rate of 20% expected at week 52 for continuous quitters after 2 weeks from AQD. This is based on an assumed increase of 5% from the 15% abstinence rate at week 52 expected for subjects at enrolment [2]. Sample size calculations are conducted using SAS® version 9.2 for the 95% CI of the mean differences between paired observations (proc power onesamplemeans) [28]. The SAS® implementation of the method published by Beal [29] is adopted to estimate the probability of obtaining at most the target 95% CI of ± 1 [% pred.].

The overall enrolment and attrition rate will be monitored during the recruitment phase of the study and sample size may be adapted to ensure at least 190 successful quitters complete V17.

12.3 Analysis Populations

Descriptive summaries will be produced and analyses will be conducted in enrolled subjects who continuously abstained from smoking (quitters) for at least 13 weeks (V8), with no major protocol deviations which impact data evaluability (to be defined in the SAP). In enrolled subjects failing to continuously abstain from smoking after V8, only endpoint data assessed prior to the date of relapse will be included in the analysis.

Descriptive summaries and analysis will also be conducted in the subgroup of subjects who completed the 1-year continuous SC (completers).

12.4 Demographics and Baseline Characteristics

Demographic and other Baseline characteristics will be summarized as reported in section 12.1.3 for the groups of quitters retained in the study at V8, V11, and V17. There will be no formal comparison of Baseline data, that is, no statistical hypothesis testing will be performed.

12.5 Study Endpoint(s)

12.5.1 Study Endpoint Analysis Variables

See section 3 “Study Objectives and Endpoints”.

12.5.2 Baseline Comparability

Study endpoints at baseline will be summarized as reported in section 12.1.3 for the quitters in the analysis population and in the subgroup of completers. There will be no formal comparison of endpoint values at Baseline, that is, no statistical hypothesis testing will be performed.

12.5.3 Descriptive Summary

Study endpoints across time will be summarized by study visit as reported in section 12.1.3 for the analysis population and in the subgroup of completers. Summary statistics of endpoints will also include the mean change from Baseline, together with 95% CI. A similar approach will be used to analyze the relative change from Baseline for those BoExp having at least 50% of the measured values above the limit of quantification (LOQ).

12.5.4 Exploratory Analysis: Random Coefficient Modeling

For clinical risk endpoints associated with CVD, respiratory diseases, airway inflammation, genotoxicity and xenobiotics, the pattern and form of the endpoint change over time will be modeled using random measurement effects models in the analysis population and in the subgroup of completers. Sex, cigarette consumption at Baseline, age, region, and other Baseline variables will be considered for inclusion in the model. A backward elimination procedure will be used to identify independent predictors of risk markers (to be defined in the SAP). Goodness of fit will be evaluated by the analysis of residuals (model diagnostics) and by comparing the values predicted versus the observed parameter changes (calibration). Least square estimates of the effect of variables included in the model will be reported together with the 95% CI.

12.5.5 Confirmatory Analyses

No confirmatory analysis is foreseen in this study although data will be used to evaluate the effect of SC for benchmarking purposes in the context of PMI products assessment programs. The details of the methods used to evaluate the reference effect of SC for specific endpoints and population characteristics will be provided in separate analysis plans, and results will be reported separately.

12.6 Safety Endpoint(s)

In general, all safety data will be listed by region for the overall screened population, and tabulated by region for the enrolled population using the approach described in section 12.1.3.

Safety summaries will also be produced for overall, and by region from V2 to V18 for the enrolled population.

Adverse event data will serve as the primary assessment of safety. Other safety variables monitored in this study include: vital signs (systolic and diastolic blood pressure, pulse rate, respiratory rate), ECG data, clinical chemistry, hematology, concomitant medications, and urine analysis safety panel, physical examination.

The number and percentage of subjects with AEs and SAEs will be tabulated by Medical Dictionary for Regulatory Activities (MedDRA) system organ class and preferred term. Summaries will also be presented for AEs leading to withdrawal, AEs leading to death, AEs by severity, and laboratory AEs. Tabulations will be performed for both the number of subjects experiencing an event and the number of events.

Descriptive statistics will be summarized for laboratory parameters, ECG, respiratory symptoms, and vital signs. The number and percentage of subjects with clinical findings will be summarized and shift tables showing change from Baseline of clinical findings will be provided for: ECGs, physical examinations and laboratory parameters (both shifts in normal ranges and toxicity grades).

12.7 Interim Analysis

No formal interim analysis of the data is foreseen. Data monitoring will be performed according to CRO's SOPs and as per the agreed monitoring plan with PMI.

Interim data sets will be extracted for the purpose of providing data for the design and interpretation of assessment studies of PMI candidate MRTPs. For this purpose, the analysis methods are detailed in a separate SAP.

13 ADMINISTRATIVE CONSIDERATIONS

13.1 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 written in the information provided to the subject. An agreement to disclose any such information will be obtained from the subject in writing and signed by the subject, in compliance with all local and national data protection and privacy legislation.

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

The blood samples for transcriptomics & lipidomics, and the data related to these samples will be anonymized. Anonymized data and samples are initially single or double coded where the link between the subjects' identifiers and the unique code(s) is subsequently deleted. This is applicable for the blood bio-banking for transcriptomics & lipidomics only.

Any documents that allow full identification of the subject (*e.g.*, the subject's signed ICF) must be maintained in confidence 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, 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.

13.2 Access to Source Documentation

Subjects will be informed that, during 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 national and local data protection and privacy legislation.

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

13.3 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 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 [30].

Essential documents must be retained by the Investigator for a minimum of:

- At least 15 years after completion or discontinuation of the study.

These documents should be retained for a longer period, however, if required by the applicable regulatory requirements or by an agreement with the Sponsor.

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 enrolment 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, the 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).
- 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.

It is the responsibility of the Sponsor to inform the Investigator/study site as to when these documents no longer need to be retained.

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

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

The Investigator or designee must obtain written approval from the Sponsor before destruction of any records. Normally, these records will be held in the Investigator's archives. If an Investigator or designee is unable to meet this obligation, they must ask the Sponsor for permission to make alternative arrangements. Details of these arrangements must be documented.

The Sponsor or Sponsor's authorized representative will maintain documentation relating to the study for 15 years after the CSR has been finalized.

13.4 Clinical Study Report

The Sponsor must 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. In certain circumstances, an abbreviated CSR may be acceptable. Submission of the CSR to the IRB/IEC will be complied with as requested by local requirements.

The results of the additional variables will be presented in the study CSR..

13.5 Financial Disclosure

Investigators and designees are required to provide financial disclosure information to the Sponsor. In addition, the Investigators and designees must provide the Sponsor with a

commitment 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.

13.6 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. Disclosure of the content of this document is allowed only to study personnel, IRB/IEC, or duly authorized representatives of regulatory agencies for this purpose 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, prompt notice will be given to the Sponsor prior to any such disclosure.

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

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Appendix 1 Study Assessments

Visits Assessments (± time window in days)	Screening	Baseline		AQD	Smoking Abstinence Period [m]														Safety Follow-Up Period
	V1	V2	V3		V4 (±3)	V5 (±3)	V6 (±8)	V7 (±8)	V8 (±8)	V9 (±8)	V10 (±8)	V11 (±8)	V12 (±8)	V13 (±8)	V14 (±8)	V15 (±8)	V16 (±8)	V17 (±8)	ET	V18 (±3)
					W1	W2	W4	W9	W13	W17	W22	W26	W30	W35	W39	W43	W48	W52		W56
ICF main study and additional ICFs	•																			
Information on the risk of smoking / smoking cessation advice [a]	•	•																		
Inclusion/exclusion criteria	•	•																		
Enrolment		•																		
Individual TQD [b]		•																		
Individual AQD [c]				•																
Smoking cessation support [d]		•	•		•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	

Visits Assessments (± time window in days)	Screening	Baseline		AQD	Smoking Abstinence Period [m]														Safety Follow-Up Period
	V1	V2	V3		V4 (±3)	V5 (±3)	V6 (±8)	V7 (±8)	V8 (±8)	V9 (±8)	V10 (±8)	V11 (±8)	V12 (±8)	V13 (±8)	V14 (±8)	V15 (±8)	V16 (±8)	V17 (±8)	ET	V18 (±3)
					W1	W2	W4	W9	W13	W17	W22	W26	W30	W35	W39	W43	W48	W52		W56
Self-reporting by the subject on continuous smoking abstinence					•	•	•	•	•	•	•	•	•	•	•	•	•	•		•[n]
Demographics	•																			
Medical history	•																			
Concomitant diseases	•																			
Prior/concomitant medication	•	•	•		•	•	•	•	•	•	•	•	•	•	•	•	•	•		
B: HIV, hepatitis B and C	•																			
U: Drug screen	•																			
U: Cotinine test (cut-off ≥ 200 ng/mL)	•	•																		

Visits Assessments (± time window in days)	Screening	Baseline		AQD	Smoking Abstinence Period [m]														Safety Follow-Up Period
	V1	V2	V3		V4 (±3)	V5 (±3)	V6 (±8)	V7 (±8)	V8 (±8)	V9 (±8)	V10 (±8)	V11 (±8)	V12 (±8)	V13 (±8)	V14 (±8)	V15 (±8)	V16 (±8)	V17 (±8)	ET	V18 (±3)
					W1	W2	W4	W9	W13	W17	W22	W26	W30	W35	W39	W43	W48	W52		W56
U: Cotinine test (cut-off < 100 ng/mL)											•	•	•	•	•	•	•	•		
Alcohol U or breath test	•																			
U: Pregnancy test (all females)	•	•			•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	
B/U: Clinical chemistry, hematology, urine analysis [e]	•	•							•			•						•	•	
ECG	•											•						•	•	
Vital signs [f]	•	•			•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	
Weight	•	•							•			•						•		
Height	•																			
Waist circumference		•							•			•						•		

Visits Assessments (± time window in days)	Screening	Baseline		AQD	Smoking Abstinence Period [m]														Safety Follow-Up Period
	V1	V2	V3		V4 (±3)	V5 (±3)	V6 (±8)	V7 (±8)	V8 (±8)	V9 (±8)	V10 (±8)	V11 (±8)	V12 (±8)	V13 (±8)	V14 (±8)	V15 (±8)	V16 (±8)	V17 (±8)	ET	V18 (±3)
					W1	W2	W4	W9	W13	W17	W22	W26	W30	W35	W39	W43	W48	W52		W56
Physical examination	•	•							•			•						•	•	
CO breath test [g]		•			•	•	•	•	•	•	•	•	•	•	•	•	•	•		
Spirometry pre- and post-bronchodilator [h]	•	•							•			•						•	•	
Lung volumes (at selected sites) [h]		•							•			•						•	•	
B: COHb		•							•			•						•		
U: Full list of BoExp [i]		•							•											
U: Reduced list of BoExp [i]												•						•		
B: CYP2A6		•							•			•						•		
B: Nicotine / cotinine		•							•			•						•		

Visits Assessments (± time window in days)	Screening	Baseline		AQD	Smoking Abstinence Period [m]														Safety Follow-Up Period
	V1	V2	V3		V4 (±3)	V5 (±3)	V6 (±8)	V7 (±8)	V8 (±8)	V9 (±8)	V10 (±8)	V11 (±8)	V12 (±8)	V13 (±8)	V14 (±8)	V15 (±8)	V16 (±8)	V17 (±8)	ET	V18 (±3)
					W1	W2	W4	W9	W13	W17	W22	W26	W30	W35	W39	W43	W48	W52		W56
B: CVD clinical risk endpoints [j]		•							•			•						•		
U: CVD clinical risk endpoints [j]		•							•			•						•		
FTND questionnaire		•																		
Cough VAS questionnaire		•							•			•						•		
Prochaska's questionnaire	•																			
Questions about smoking history/habits	•	•																		
SES questionnaire [k]		•																		
Lifestyle questions	•	•							•			•						•		
AE/SAE recording	•	•	•		•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•

Visits Assessments (± time window in days)	Screening	Baseline		AQD	Smoking Abstinence Period [m]														Safety Follow-Up Period
	V1	V2	V3		V4 (±3)	V5 (±3)	V6 (±8)	V7 (±8)	V8 (±8)	V9 (±8)	V10 (±8)	V11 (±8)	V12 (±8)	V13 (±8)	V14 (±8)	V15 (±8)	V16 (±8)	V17 (±8)	ET	V18 (±3)
					W1	W2	W4	W9	W13	W17	W22	W26	W30	W35	W39	W43	W48	W52		W56
B: bio-banking for transcriptomics / lipidomics [l]		•							•			•						•		
B: bio-banking for clinical risk endpoints and BoExp [l]		•							•			•						•		
U: bio-banking for Clinical risk endpoints and BoExp [l]		•							•			•						•		

Abbreviations: AE = Adverse event; AQD = Actual quit date; B = Blood sample required; BoExp = Biomarkers of exposure; CO = Carbon monoxide; COHb = Carboxyhemoglobin; CVD = cardiovascular disease; CYP = Cytochrome P450; ECG = Electrocardiogram; ET = Early Termination; FTND = Fagerström test for nicotine dependence; HIV = Human immunodeficiency virus; ICF = Informed consent form; SAE = Serious adverse event; SES = Socio-economic situation; TQD = Target quit date; U = Urine sample required; V = Visit; VAS = Visual analog scale; W = Week.

a. At V2, advices on the risk of smoking and smoking cessation advice should be the last assessment of the day

b. At V2, subjects will be asked to define their TQD. This TQD has to be within 14 days after the check-out of V2 and V3 has to be within 24 to 48 hours after TQD

c. Subjects will record their AQD which has to be within 14 days after the TQD. Of note, the AQD is not a visit

d. Smoking cessation support including smoking cessation counseling and behavioral support throughout the study according to the smoking cessation support plan. Additional SC support will be offered at any time when requested by the subject

e. The list of clinical chemistry, hematology and urine analysis parameters are detailed Table 2.

f. Systolic and diastolic blood pressure, pulse and respiratory rate.

g. CO breath test will be done with a smokerlyser or similar

h. The lung function tests must be performed in the following sequence when appropriate:

- Spirometry without salbutamol
- Lung volume (VC, TLC, and IC)*
- Spirometry with salbutamol

* Lung volumes will be assessed in selected center(s) specialized in lung function testing

i. Full and reduced lists of urinary BoExp are provided in Appendix 5. Free cotinine concentration (part of the nicotine equivalents) will be determined in 24-hour urine collected at Visit 11 (cut-off ≥ 50 ng/mL) as a criterion for discontinuation at the time the results are made available.

j. List of clinical risk endpoints assessed in the different matrices is provided in Appendix 4

k. A country specific socio-economic situation questionnaire will be completed in the following countries: US, UK, Poland, Germany and Japan

l. Only in subjects who signed an additional ICF

m. For the visits, a time window of ± 8 days is allowed from the AQD, with the exception of V4 and V5 (± 3 days)

n. Self-reporting by the subject on continuous smoking abstinence at Visit 18 (Week 56) is performed via phone contact if the subject has previously completed V17.

Appendix 2 Medications with Impact on Clinical Risk Endpoints (with Half-Lives)

Drug	Biological or Elimination Half-life (h)	Last Dose Applied in hours Prior to Lab Value Tested (5 x half-life)	Impact on Endpoint
Vitamins			
Pyridoxin (vitamin B6)	2 - 5	10 - 25	HDL-C
Cyanocobalamin (vitamin B12)	approx. 24 - 48	120 - 240 (≈ 5 - 10 days)	HDL-C
Thiamine chlorid (vitamin B1)	4 - 6	20 - 30	HDL-C
Nicotinic acid (vitamin B3)	approx. 24	120 (≈ 5 days)	HDL-C
Ascorbicacid (vitamin C)	2.9	14.5	HDL-C
Alpha-Tocopherol (vitamin E)	approx. 5 - 7 days	approx. 25 - 35 days	$PGF_{2\alpha}$
Systemic and Inhaled Drugs Affecting Spirometry			
Anticholinergica/Antimuscarinica			
Ipratropiumbromide	3.6	18	FEV_1
Tiotropium	120 - 144 (≈ 5 - 6 days)	600 - 720 (≈ 25 - 30 days)	FEV_1
Aclidiniumbromid	2 - 3	10 - 15	
Short-acting β-agonists, orally taken			
Terbutalinsulfat	Plasma $t_{1/2}$: 3 - 4	Plasma $t_{1/2}$: 15 - 20	FEV_1
Bambuterol	22 - 24	110 - 120 (≈ 4.6 - 5 days)	FEV_1
Inhaled short-acting β-agonists			

Drug	Biological or Elimination Half-life (h)	Last Dose Applied in hours Prior to Lab Value Tested (5 x half-life)	Impact on Endpoint
Salbutamolsulfat	Plasma $t_{1/2}$: 2.7 - 5	Plasma $t_{1/2}$: 13.5 - 25	FEV_1
Fenoterol	approx. 3	approx. 15	FEV_1
Inhaled long-acting β-agonists			
Formoterolfumarat	approx. 5	approx. 25	FEV_1
Indacaterolmaleat	40 - 52	200 - 260 (≈ 8.3 - 10.8 days)	FEV_1
Salmeterol	n.a. no systemic effect	n.a	FEV_1
Inhaled Glucocorticosteroids			
Beclomethasone	2.8	14	FEV_1
Budesonide	2 - 3	10 - 15	FEV_1
Ciclesonide	6 - 7	30 - 42 (≈ 1.25 - 1.75 days)	FEV_1
Fluticasonpropionate	16 - 21.3	80 - 106.5 (≈ 3.3 - 4.4 days)	FEV_1
Systemic Broncholytic Drugs			
Montelukast	Plasma $t_{1/2}$: 2,7 – 5,5	13.5 - 27.5	FEV_1
Aminophyllin, Theophyllin	7 - 9	35 - 45	FEV_1
Roflumilast	Plasma $t_{1/2}$: 17	85 (≈ 3.5 days)	FEV_1
β-blockers			
Atenolol	6 -10	30 - 50 (≈1.25 - 2 days)	FEV_1
Metoprolol	3 - 5	15 - 25	FEV_1
Bisoprolol	17 ± 5	85 (≈3.5 days)	FEV_1
Antiplatelet Agents			
Phenprocoumon	165 (≈ 6.9 days)	825 (≈ 34 days)	$PGF_{2\alpha}$

Drug	Biological or Elimination Half-life (h)	Last Dose Applied in hours Prior to Lab Value Tested (5 x half-life)	Impact on Endpoint
Apixaban	12	60 (≈ 2.5 days)	$PGF_{2\alpha}$
Warfarin	Plasma $t_{1/2}$: approx. 35 - 45	175 - 225 (≈ 7.3 - 9.4 days)	$PGF_{2\alpha}$
Rivaroxaban	5 - 13 (age - dependent)	25 - 65 (≈ 1 - 2.7 days)	$PGF_{2\alpha}$
Acetylsalicylic acid	2 - 30 (dose - dependent)	10 - 150 (up to 6.25 days)	$PGF_{2\alpha}$
Antidiabetic Drugs			
Acarbose	9,6 ± 4,4	48 (≈ 2 days)	11-DTX-B2
Metformin	6.5	32.5 (≈ 1.3 days)	11-DTX-B2
Vildagliptin	3	15	11-DTX-B2
Saxagliptin	26.9	134.5 (≈ 5.6 days)	11-DTX-B2
Repaglinid	4 - 6	20 - 30 (up to 1.25 days)	11-DTX-B2
Glibenclamid	8 - 10	40 - 50 (≈ 1.7 - 2.1 days)	11-DTX-B2
Glimepirid	5 - 8	20 - 40 (up to 1.7 days)	11-DTX-B2
Gliclazid	12 - 20	60 - 100 (≈ 2.5 - 4.2 days)	11-DTX-B2
Pioglitazon	16 - 23	80 - 115 (≈ 3.3 - 4.8 days)	HDL-C
NSAIDs			
Celecoxib	8 - 12	40 - 60 (≈ 1.7-2.5 days)	WBC, 11-DTX-B2
Etoricoxib	22	110 (≈ 4.6 days)	WBC, 11-DTX-B2
Diclofenac	approx. 2	approx. 10	WBC, 11-DTX-B2
Indometacin	2; terminal phase: 4 - 11	10; terminal phase: 20-55 (up to 2.3 days)	WBC, 11-DTX-B2

Drug	Biological or Elimination Half-life (h)	Last Dose Applied in hours Prior to Lab Value Tested (5 x half-life)	Impact on Endpoint
Meloxicam	approx. 20	approx. 100 (≈ 4.2 days)	WBC, 11-DTX-B2
Piroxicam	approx. 50	250 (≈ 10.4 days)	WBC, 11-DTX-B2
Ibuprofen	1.8 - 3.5	9 - 17.5	WBC, 11-DTX-B2
Ketoprofen	1.5 - 2.5 (up to 8)	7.5 - 12.5 (up to 40)	WBC, 11-DTX-B2
Naproxen	10 - 18	50 - 90 (≈ 2.1 - 3.75 days)	WBC, 11-DTX-B2
Angiotensin-Converting Enzyme Inhibitors			
Enalapril	at least 24	at least 120 (at least 5 days)	11-DTX-B2
Quinapril	approx. 26	approx. 130 (≈ 5.4 days)	11-DTX-B2
Ramipril	13 - 17	65 - 85 (≈ 2.7 - 3.5 days)	11-DTX-B2
Lisinopril	12.6	63 (≈ 2.6 days)	11-DTX-B2
Antidepressant Drugs			
Bupropion	20	100 (≈ 4.2 days)	WBC
Selective serotonin reuptake inhibitors (SSRIs)			
Citalopram	35	175 (≈ 7.29 days)	WBC
Escitalopram	27 – 32	135 – 160 (up to 6.67 days)	WBC
Fluoxetine	Initial: 24 - 72 Chronic: 96 – 144	Initial: 120 – 360 (up to 15 days) Chronic: 480 – 720 (up to 30 days)	WBC
Fluvoxamine	16	80 (≈ 3.33 days)	WBC
Paroxetine	21	105 (≈ 4.38 days)	WBC
Sertraline	26	130 (≈ 5.42 days)	WBC

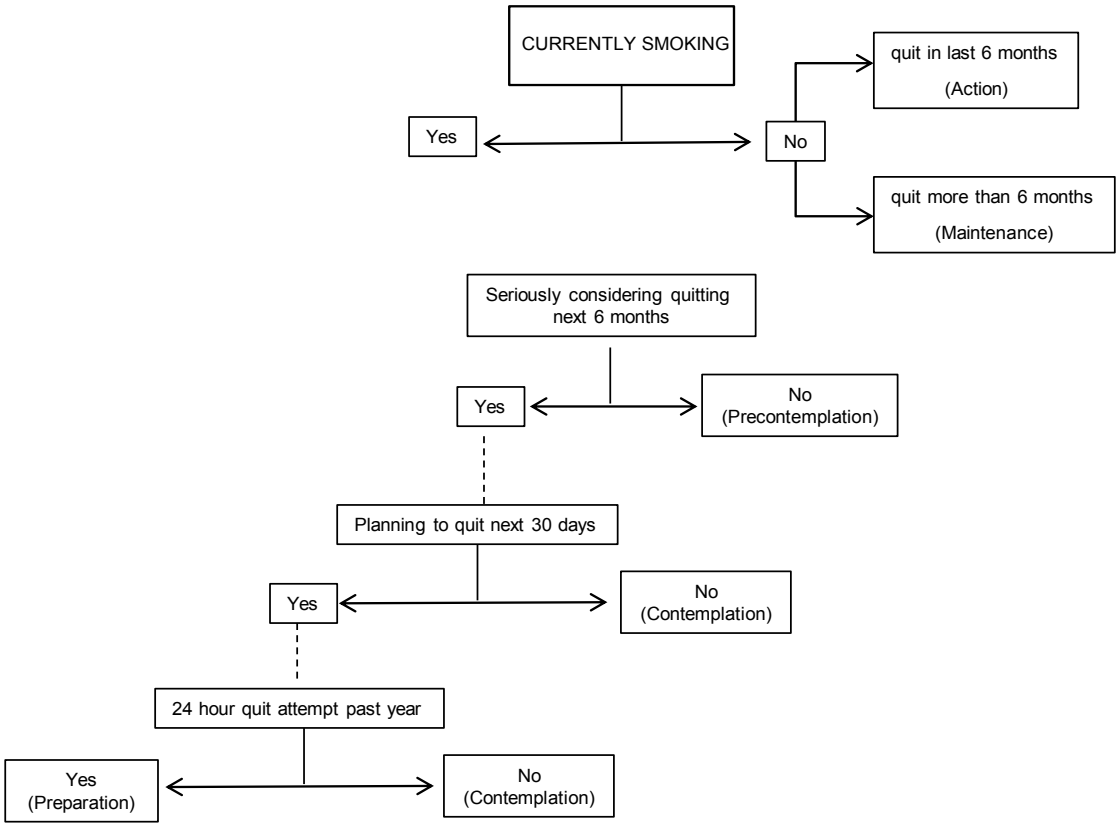
Drug	Biological or Elimination Half-life (h)	Last Dose Applied in hours Prior to Lab Value Tested (5 x half-life)	Impact on Endpoint
Serotonin–norepinephrine reuptake inhibitors (SNRIs)			
Venlafaxine	5 ± 2	25 ± 10 (≈ 1.0 ± 0.4 days)	WBC
Tricyclic antidepressants			
Tricyclic clomipramine	20 - 26	100 - 130 (≈ 4.2 - 5.4 days)	WBC
Non-specified antidepressant drug class			
Nefazodone	11 - 24	55 - 120 (≈ 2.3 - 5 days)	WBC
Lipid Lowering Drugs			
Fibrate			
Bezafibrat	1 - 2	5 - 10	HDL-C, Total Cholesterol, Triglycerides
Fenofibrat	Plasma $t_{1/2}$ approx. 20	approx. 100 (≈ 4.2 days)	HDL-C, Total Cholesterol, Triglycerides
Gemfibrozil	1.3 - 1.5	6.5 - 7.5	HDL-C, Apolipoproteins
Bile acid sequestrants			
Colestyramin	no absorption into blood stream	n.a.	HDL-C
Statines			
Atorvastatin	20 - 30	100 - 150 (≈ 4.2 - 6.25 days)	HDL-C, 11-DTX-B2
Simvastatin	approx. 2-3	10 - 15	HDL-C, 11-DTX-B2
Rosuvastatin	approx. 19	95	HDL-C, 11-DTX-B2

Drug	Biological or Elimination Half-life (h)	Last Dose Applied in hours Prior to Lab Value Tested (5 x half-life)	Impact on Endpoint
Fluvastatin	2.3 ± 0.9	11.5 ± 4.5	HDL-C, 11-DTX-B2
Other cholesterol-lowering drugs			
Ezetimibe	22	110 (≈ 4.58 days)	HDL-C, 11-DTX-B2, Apolipoproteins, Total Cholesterol, Triglycerides

* n.a. = not applicable

Appendix 3 Prochaska "Stage of Change" Questionnaire

The Prochaska questionnaire is structured as follows:



Appendix 4 Summary of Clinical Risk Endpoints

Clinical Risk Endpoints	Measurement of	Biofluid / Function
Respiratory		
Forced expiratory volume in 1 second (FEV_1)	Spirometry	
Forced vital capacity (FVC)	Spirometry	
Forced expiratory flow 25-75 (FEF 25-75)	Spirometry	
Total lung volumes, inspiratory capacity and functional residual capacity#	Lung volume	Lung
Frequency / intensity of cough symptoms	Lung response (respiratory symptoms)	
Cardiovascular		
High density lipoprotein cholesterol (HDL-C)	Lipid metabolism	Serum
Low density lipoprotein cholesterol (LDL-C)	Lipid metabolism	Serum
White blood cell count (WBC)*	Inflammation	Blood
High sensitivity C-reactive protein (hs-CRP)	Inflammation	Serum
Platelet count*	Inflammation	Blood
Fibrinogen	Inflammation	Plasma
Soluble intercellular adhesion molecule-1 (sICAM-1)	Endothelial dysfunction	Serum
11-dehydrothromboxane B2 (11-DTX-B2)	Platelet activation	Urine
Albumin	Endothelial dysfunction	Urine
Homocysteine	Endothelial dysfunction	Plasma
8-epi-prostaglandin F2α (8-epi-$PGF_{2\alpha}$)	Oxidative stress	Urine
Myeloperoxidase (MPO)	Oxidative stress	Serum
Apolipoprotein A1 (Apo A1)	Lipid metabolism	Serum
Apolipoprotein B (Apo B)	Lipid metabolism	Serum
Carboxyhemoglobin (COHb)	Transport of oxygen by hemoglobin	Blood
Glycosylated hemoglobin (HbA1c)	Insulin resistance	Blood

Clinical Risk Endpoints	Measurement of	Biofluid / Function
Xenobiotics		
Cytochrome 2A6 (CYP2A6) activity	Nicotine metabolism	Plasma
Genotoxicity		
Total 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (Total NNAL)	Exposure to carcinogenic HPHC (NNK)	Urine

* Measured as part of the safety laboratory parameters.

Measured in selected centers.

Appendix 5 Summary of Biomarkers of Exposure to HPHC

HPHC [smoke phase]	HPHC list	Biomarker	Matrix	$t_{1/2\beta}$	Reduced List of BoExp
1,3-Butadiene [gas]	FDA-18, FDA-93, HC PMI-58, WHO-18	Monohydroxybutenylmercapturic acid (MHBMA)	Urine	4 to 16 h	•
1-Aminonaphthalene [particulate]	FDA-18, FDA-93, HC PMI-58	1-Aminonaphthalene (1-NA)	Urine	Not described	
2-Aminonaphthalene [particulate]	FDA-18, FDA-93, HC PMI-58, WHO-18	2-Aminonaphthalene (2-NA)	Urine	9 h	
4-Aminobiphenyl [particulate]	FDA-18, FDA-93, HC PMI-58, WHO-18	4-Aminobiphenyl (4-ABP)	Urine	26 h	
Acrolein [gas]	FDA-18, FDA-93, HC PMI-58, WHO-18	3-Hydroxypropyl-mercapturic acid (3-HPMA)	Urine	10 h	•
Acrylonitrile [gas]	FDA-18, FDA-93, HC PMI-58, WHO-18	2-Cyanoethylmercapturic acid (CEMA)	Urine	17 h	•
Benzene [gas]	FDA-18, FDA-93, HC PMI-58, WHO-18	S-Phenyl-mercapturic acid (S-PMA)	Urine	9 to 15 h	
Benzo[a]pyrene [particulate]	FDA-18, FDA-93, HC PMI-58, WHO-18	3-Hydroxybenzo[a]pyrene B[a]P	Urine	3 to 4 h	•
Carbon monoxide [gas]	FDA-18 FDA-93 HC	CO	Breath	/	•

HPHC [smoke phase]	HPHC list	Biomarker	Matrix	$t_{1/2\beta}$	Reduced List of BoExp
	PMI-58 WHO-18				
Pyrene	FDA-18 FDA-93 HC PMI-58 WHO-18	Total 1-hydroxypyene (total 1-OHP)	Urine	6 to 35 h	•
Crotonaldehyde [gas]	FDA-18, FDA-93, HC PMI-58, WHO-18	3-Hydroxy-1-methylpropyl-mercapturic acid (3-HMPMA)	Urine	2 days	•
Ethylene oxide [gas]	FDA-93, PMI-58	2-Hydroxyethyl-mercapturic acid (HEMA)	Urine	5 h	
NNN [particulate]	FDA-18, FDA-93, HC PMI-58, WHO-18	Total N-nitrosonornicotine (Total NNN)	Urine	15 h	•
o-Toluidine [gas]	FDA-93, PMI-58	o-Toluidine (o-TOL)	Urine	10 to 16 h	
Toluene [gas]	FDA-18, FDA-93, HC PMI-58, WHO-18	S-benzyl-mercapturic acid (S-BMA)	Urine	9 h	
Nicotine [particulate]	FDA-18, FDA-93, HC PMI-58	Nicotine (NIC-P)	Plasma	1 to 2 h	•
		Cotinine (COT-P) 3-OH Cotinine (3-OHCOTP)	Plasma	16 to 18 h -	•
		Nicotine equivalents (Neq)	Urine	16 h (estimated)	•

Appendix 6 Abnormal Laboratory Values

ABNORMAL LABORATORY VALUES RATING: SERUM CHEMISTRY PARAMETERS

Serum Chemistry*	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**	**Life-threatening (Grade 4)**
Sodium – Hyponatremia (mmol/L) (1)	<LLN - 130	-	<130 - 120	<120
Sodium – Hypernatremia (mmol/L) (1)	>ULN - 150	>150 - 155	>155 - 160; hospitalization indicated	>160
Potassium – Hyperkalemia (mmol/L)(1)	>ULN - 5.5	>5.5 - 6.0	>6.0 -7.0; hospitalization indicated	>7.0
Potassium – Hypokalemia (mmol/L) (1)	<LLN - 3.0	<LLN - 3.0; symptomatic; intervention indicated	<3.0 - 2.5; hospitalization indicated	<2.5
Glucose – Hypoglycemia (1) (mg/dL) (mmol/L)	 <LLN – 55; <LLN – 3.0	 <55 – 40; <3.0 – 2.2	 <40 – 30; <2.2 – 1.7	 <30; <1.7
Glucose – Hyperglycemia: (1) Fasting (mg/dL) (mmol/L)	 >ULN-160; >ULN-8.9	 >160-250; >8.9-13.9	 >250-500; >13.9-27.8; hospitalization indicated	 >500; >27.8
Creatinine increased (1)	>1 – 1.5 x Baseline; >ULN – 1.5 x ULN	>1.5 – 3.0 x Baseline; >1.5 – 3.0 x ULN	>3.0 x Baseline; >3.0 – 6.0 x ULN	>6.0 x ULN
Albumin - Hypoalbuminemia (1) (g/dL)	<LLN – 3;	<3 – 2;	<2;	-

(g/L)	<LLN - 30	<30 - 20	<20	
Alkaline phosphatase increased (1)	>ULN – 2.5 x ULN	>2.5 – 5.0 x ULN	>5.0 – 20.0 x ULN	>20.0 x ULN
ALT / AST increased (1)	>ULN – 3.0 x ULN	>3.0 – 5.0 x ULN	>5.0 – 20.0 x ULN	>20.0 x ULN
Gamma-glutamyl transferase (GGT) increased (1)	>ULN – 2.5 x ULN	>2.5 – 5.0 x ULN	>5.0 – 20.0 x ULN	>20.0 x ULN
Blood bilirubin increased (total and direct) (1)	>ULN – 1.5 x ULN	>1.5 – 3.0 x ULN	>3.0 – 10.0 x ULN	>10.0 x ULN
Cholesterol high (1)				
(mg/dL) (mmol/L)	>ULN - 300; >ULN - 7.75	>300-400; >7.75-10.34	>400-500; >10.34-12.92	>500; >12.92
Triglycerides - Hypertriglyceridemia (1)				
(mg/dL) (mmol/L)	150 – 300; 1.71 – 3.42	>300 – 500; >3.42 – 5.70	>500 – 1000; >5.70 – 11.40	>1000; >11.4

Abbreviations: ALT = alanine aminotransferase; AST = aspartate aminotransferase; BUN = blood urea nitrogen; GGT = gamma-glutamyl transferase; LLN = lower limit of the normal range; ULN = upper limit of the normal range.

Data Sources:

(1) Common Terminology Criteria for Adverse Events (CTCAE) version 4.03.Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials, U.S. Department of Health and Human Services, FDA, Center for Biologics Evaluation and Research - Guidance for Industry.

* Those parameters that are not listed and do not have grading categories in the CTCAE will be reviewed by the Investigator or designne, and will only be reported as an AE if considered to be clinically relevant.

ABNORMAL LABORATORY VALUES RATING: HEMATOLOGY PARAMETERS

Hematology*	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**	**Life-threatening (Grade 4)**
Anemia (Hemoglobin) (g/dL) (mmol) g/L	<LLN-10.0 <LLN-6.2 < 100	<10-8.0 <6.2-4.9 < 100-80	<8.0 <4.9 <80 Transfusion indicated	Life threateming conseuqences; urgent intervention indicated
Hemoglobin increase – (g/dL) (1)	Increase in >0 – 2 above ULN or above Baseline if Baseline is above ULN	Increase in >2 – 4 above ULN or above Baseline if Baseline is above ULN	Increase in >4 above ULN or above Baseline if Baseline is above ULN	-
WBC Decrease - (cell/mm^3) (1) 10^{-9}/l	<LLN – 3000; <LLN – 3.0	<3000 - 2000; <3.0 – 2.0	<2000 - 1000; <2.0 – 1.0	<1000; <1.0
Lymphocytes Increase - (cell/mm^3) (1)	-	>4,000 – 20,000	>20,000	-
Lymphocytes Decrease – (cell/mm^3) (1)	<LLN – 800; <LLN – 0.8 x 10^{-9}/l	<800 - 500; <0.8 – 0.5 x 10^{-9}/l	<500 - 200; <0.5 – 0.2 x 10^{-9}/l	<200; <0.2 x 10^{-9}/l
Neutrophils Decrease - (cell/mm^3) (1)	<LLN – 1500; <LLN – 1.5 x 10^{-9}/l	<1500 - 1000; <1.5 – 1.0 x 10^{-9}/l	<1000 - 500; <1.0 – 0.5 x 10^{-9}/l	<500; <0.5 x 10^{-9}/l
Platelets Decrease - (cell/mm^3) (1)	<LLN – 75,000; <LLN – 75.0 x 10^{-9}/l	<75,000 – 50,000; <75.0 – 50.0 x 10^{-9}/l	<50,000 – 25,000; <50.0 – 25.0 x 10^{-9}/l	<25,000; <25.0 x 10^{-9}/l

Abbreviations: LLN = lower limit of the normal range; ULN = upper limit of the normal range; WBC = white blood cell.

Data Source:

(1) Common Terminology Criteria for Adverse Events (CTCAE) version 4.03.

* Those parameters that are not listed and do not have grading categories in the CTCAE will be reviewed by the Investigator or designee, and will only be reported as an AE if considered to be clinically relevant.

ABNORMAL LABORATORY VALUES RATING: URINALYSIS PARAMETERS

Urine*	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Life-threatening (Grade 4)
Protein (1)	1+ proteinuria; urinary protein <1.0 g/24 hours	2+ proteinuria; urinary protein 1.0-3.4 g/24 hours	Urinary protein ≥3.5 g/24 hours	-

Abbreviations: ADL = activities of daily living; IV = intravenous.

Data Source:

(1) Common Terminology Criteria for Adverse Events (CTCAE) version 4.03.

* Those parameters that are not listed and do not have grading categories in the CTCAE will be reviewed by the Investigator or designee, and will only be reported as an AE if considered to be clinically relevant.