AN EXPLORATORY PET-STUDY OF DEPOSITION, DISPOSITION AND BRAIN UPTAKE OF [^{11}C]NICOTINE AFTER INHALATION OF 2 NICOTINE FORMULATIONS VIA THE *MY*BLUTM E-CIGARETTE IN SMOKERS

NCT# NCT04780815

Study Protocol - FINAL v2.0; 30JAN2020

Clinical Study Protocol	
Investigational Product	*my*blu™ e-cigarette system
Sponsor study code	NER 03/001
Protocol Version and Date	FINAL v2.0; 30JAN2020

AN EXPLORATORY PET-STUDY OF DEPOSITION, DISPOSITION AND BRAIN UPTAKE OF [^{11}C]NICOTINE AFTER INHALATION OF 2 NICOTINE FORMULATIONS VIA THE *MY*BLU™ E-CIGARETTE IN SMOKERS

Test product and dose	*my*blu™ e-cigarette system Formulation 1: 2.5% freebase nicotine formulation (no flavour) Formulation 2: 2.5% Nicotine salt formulation (no flavour)
Sponsor signatory	[redacted] Nerudia Ltd, Wellington house Physics road, Speke Liverpool, L24 9HP United Kingdom
Principal Investigator	[redacted] CTC Clinical Trial Consultants AB Uppsala University Hospital, Entrance 85, 2nd level SE-751 85 Uppsala, Sweden
Clinical study conduct and management	CTC Clinical Trial Consultants AB Uppsala University Hospital, Entrance 85, 2nd level SE-751 85 Uppsala, Sweden PET Centre, Uppsala University Hospital, Entrance 70, 1st floor, SE-751 85 Uppsala, Sweden CTC Clinical Trial Consultants AB Dag Hammarskjölds väg 10B SE-752 37 Uppsala, Sweden

1 STUDY SYNOPSIS

Study title

An exploratory PET-study of deposition, disposition and brain uptake of [^{11}C]nicotine after inhalation of 2 nicotine formulations via the *my*blu™ e-cigarette in smokers

Study code

NER 03/001

Planned study period

Q1 2020 to Q2 2020

Principal Investigator

[redacted]
CTC Clinical Trial Consultants AB, Uppsala, Sweden

Study design

This is a single centre, exploratory PET-study of deposition, disposition and brain uptake of [^{11}C]nicotine when given to smokers as two different formulations via the *my*blu™ e-cigarette system.

The study is divided in two parts:

PART A: PET 1 and PET 2 (including 5 subjects/formulation, total 10 subjects)

PART B: PET 3 (including 2 subjects/formulation + 3 optional subjects, total 4-7 subjects)

Objectives

Primary objectives PART A

- To measure the amount of nicotine deposited in the lungs and the oral cavity during 40 minutes after administration of ^{11}C-labelled nicotine via the *my*blu™ e-cigarette system using PET

Secondary objectives PART A

- To describe the single-dose pharmacokinetics of ^{11}C-labelled nicotine, given via the *my*blu™ e-cigarette system, by measuring the radioactivity in arterial blood in discrete samples up to 30 minutes following administration
- To evaluate safety and tolerability of ^{11}C-labelled nicotine inhaled via the *my*blu™ e-cigarette system

Exploratory objectives PART B

- To measure the amount of nicotine deposited in the brain during 30 minutes after administration of 11C-labelled nicotine via the myblu™ e-cigarette system using PET
- To describe the single-dose pharmacokinetics of ^{11}C-labelled nicotine, given via the *my*blu™ e-cigarette system, by measuring the radioactivity in arterial blood in discrete samples up to 30 minutes following administration
- To evaluate safety and tolerability of ^{11}C-labelled nicotine inhaled via the *my*blu™ e-cigarette system

Endpoints

Primary endpoints PART A

- Dynamic PET Digital Imaging and Communications in Medicine (DICOM) images over the lungs and oral cavity 0-40 minutes after administration
- Radioactivity vs. time profiles for the lungs and oral cavity 0-40 minutes after administration

Secondary endpoints PART A

- Radioactivity vs. time profiles for arterial blood at 2, 4, 6, 8, 10, 15, 20 and 30 minutes after administration of [^{11}C]nicotine inhalation
- Frequency, intensity and seriousness of adverse events (AEs)
- Clinically significant changes in vital signs

Exploratory endpoints (PART B)

- Dynamic PET DICOM images over the brain 0-30 minutes after administration
- Radioactivity vs. time profiles for arterial blood at 2, 4, 6, 8, 10, 15, 20 and 30 minutes after administration of [^{11}C]nicotine inhalation
- Frequency, intensity and seriousness of AEs
- Clinically significant changes in vital signs

Number of subjects planned

Approximately 25 subjects will be screened to achieve a total of 14-17 included and evaluated subjects.

Diagnosis and main eligibility criteria

Male and female (of non-childbearing potential) smokers, aged 50-65 years, with a body mass index (BMI) of $\geq$ 18.0 and $\leq$ 30.0 kg/m^2 will be considered for participation in the study.

Subjects with a history of any clinically significant disease or disorder which, in the opinion of the Investigator, may either put the subject at risk because of participation in the study, or influence the results or the subject's ability to participate in the study will be excluded from participation. Subjects, who previously have been exposed to radiation in studies, their medical history or their occupation, and who are under regular medical treatment for respiratory tract disorders or who use anticoagulantia will also be excluded from participation.

For PART B, subjects with conditions contraindicating magnetic resonance imaging (MRI) will be excluded.

Methodology – PART A

In PART A of the study, two different formulations of [^{11}C]nicotine will each be given to 5 subjects. The subjects will have two PET-assessments each (PET 1 and PET 2) after inhalation of [^{11}C]nicotine via the *my*blu™ during the same treatment day. PART A will be completed for 5 subjects on each formulation before initiation of PART B.

The IP will be given as inhalation ("puffs") via the *my*blu™ e-cigarette system to obtain a tracer dose of up to 25 megabecquerel (MBq)/PET-session.

Subjects will come for 2 visits to the clinic and have 1 End-of Study follow-up telephone call.

Screening (Visit 1) will take place from Day -28 to Day -1 and will include an eligibility check, review of health status and inhalation training.

At Visit 2, subjects will arrive to the clinic in the morning of Day 1 and will remain at the clinic until the afternoon. Day 1 will contain pre-PET safety assessment, preparation for the PET assessments (including insertion of an arterial catheter), two PET assessment sessions with at least 2 hours between the sessions and safety follow-up (including removal of arterial catheter) after the second PET assessment.

The subjects will have breakfast (optional) at the research clinic before the first PET-session. Lunch will be served at the research clinic between the two PET-sessions.

The subjects will be carefully monitored by clinical staff during and after the PET-sessions. There is immediate access to equipment and qualified staff in case of emergency.

A final End-of-Study phone call (Visit 3) will take place on Day 7 (±2) or after early withdrawal to follow up on safety (AEs and concomitant medications).

Methodology – PART B

In PART B of the study, two different formulations of [^{11}C]nicotine will each be given to 2 subjects. The subjects will have one PET-assessments each (PET 3) after two inhalations of [^{11}C]nicotine via the *my*blu™.

PART B will be completed for 2 subjects on each formulation before a decision will be taken if 3 additional subjects should be added to PART B. The decision is based on whether the results from the first scans show significant, quantifiable uptake of radioactivity in the brain. If 3 additional subjects are included, they will be treated with the formulation that showed the best uptake profile.

The IP will be given as inhalation ("puffs") via the *my*blu™ e-cigarette system to obtain a tracer dose of up to 50 MBq.

Subjects will come for 2 visits to the clinic, 1 MRI assessment visit and have 1 End-of Study follow-up telephone call (Visit 3).

Screening (Visit 1) will take place from Day -28 to Day -1 and will include an eligibility check, review of health status and inhalation training.

At Visit 2, subjects will arrive to the clinic in the morning of Day 1 and will remain at the clinic until the afternoon. Day 1 will contain pre-PET safety assessments, preparation for the PET assessments (including insertion of an arterial catheter), one PET assessment session and safety follow-up (including removal of arterial catheter) after the PET assessment.

The subjects will have breakfast (optional) at the research clinic before the PET-session. Lunch (optional) will be served at the research clinic after the PET session.

The subjects will be carefully monitored by clinical staff during and after the PET-session. There is immediate access to equipment and qualified staff in case of emergency.

A final End-of-Study phone call (Visit 3) will take place on Day 7 (±2) or after early withdrawal to follow up on safety (AEs and concomitant medications).

During the study period, the subjects will also have an MRI-scan. The MRI can be performed at any suitable timepoint during the study after the screening visit until the End-of-Study-visit.

Investigational Product (IP), dosage and mode of administration

The IPs will be supplied as the *my*blu™ e-cigarette system with e-liquid pods:

The *my*blu™ e-cigarette will be supplied together with a USB charger.

E-liquid pods will be supplied as:

- Pods for training purposes, containing 2.5% freebase nicotine formulation (no flavour) corresponding to Formulation 1.
- Pods for training purposes, containing 2.5% nicotine salt formulation (no flavour) corresponding to Formulation 2.
- Empty pods (for filling at the PET Centre with radiolabelled Formulation 1 and Formulation 2)

Bulk liquid Formulation 1 and Bulk liquid Formulation 2, will be supplied, for radiolabelling and filling of empty e-liquid pods at the PET Centre.

Duration of treatment

1 day

Duration of each subject's involvement in the study

Each subject is expected to participate in the study for approximately 7 days from the day of PET-session (Day 1, Visit 2) to the End-of Study Visit. The Screening Visit will be performed within 28 days prior to PET-scanning (Day -28 to Day -1).

PET assessment

PET DICOM images over the lungs and oral cavity (PART A) and brain (PART B). Radioactivity vs. time profiles for arterial blood after administration of [^{11}C]nicotine

Safety assessments

AEs

Vital signs

Statistical methods

No formal sample size calculation has been performed for this study. The proposed sample size is considered sufficient to provide adequate information for the study objectives.

Continuous data will be presented in terms of evaluable and missing observations, arithmetic mean, standard deviation (SD), median, minimum and maximum value. Categorical data will be presented as counts and percentages. When applicable, summary data will be presented by

treatment, and by assessment time. Individual subject data will be listed by subject number, treatment, and, where applicable, by assessment time. All descriptive summaries and statistical analyses will be performed using SAS Version 9.4 or later (SAS Institute, Inc., Cary, NC). Baseline will be defined as the visit with last data collection point prior to the first administration of IP. No imputation of missing data will be performed.

The PET Centre will be responsible for evaluation of PET data, and for presenting time activity data on [^{11}C]nicotine deposition during the dynamic scans over the two body positions in PET-scan 1 and 2. For the evaluation of [^{11}C]nicotine kinetics in the lung, primary bronchi, oral cavity and airways, volumes of interest (VOIs) will be delineated, and time activity data generated utilising PET-image analysis software

For PET-scan 3 acquired over the brain, the amount of nicotine distributed to the brain will be estimated. A whole brain VOI will be delineated on the T1-MR images overlaid on the brain PET-image. This part of the study is explorative and dependent on the time course of the [^{11}C]nicotine uptake to the blood and the time activity uptake for brain nicotine may be difficult to measure with a sufficient signal-to-noise ratio.

Study reporting

A clinical study report (CSR) will be prepared after completion of the study.

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3 LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviation or term	Explanation
ADL	Activities of daily living
AE	Adverse event
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
ATC	Anatomical therapeutic chemical
Bq	Becquerel
BMI	Body mass index
CSP	Clinical study protocol
CSR	Clinical study report
CT	Computerised tomography
CTC	Clinical Trial Consultants AB
CTCAE	Common terminology criteria for adverse events
DICOM	Digital imaging and communications in medicine
DMP	Data management plan
ECG	Electrocardiogram
eCRF	Electronic case report form
EDC	Electronic data capture
EEA	European Economic Area
FAS	Full analysis set
FSH	Follicle stimulating hormone
GCP	Good clinical practice
GDPR	General data protection regulation
Hb	Haemoglobin
HIV	Human immunodeficiency virus
HPLC	High-pressure liquid chromatography
ICF	Informed consent form
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
IEC	Independent ethics committee
IME	Important medical event
IP	Investigational product

ISF	Investigator site file
mAh	Milliampere hour
MBq	Megabecquerel
MedDRA	Medical dictionary for regulatory activities
mGy	Milligray
min	minute
mL	Millilitre
MRI	Magnetic resonance imaging
mSv	millisievert
PET	Positron emission tomography
PII	Personally Identifiable Information
PK	Pharmacokinetics
PPS	Per protocol analysis set
PT	Preferred term
PV	Pharmacovigilance
QA	Quality assurance
QC	Quality control
RBC	Red blood cell
RBM	Risk based monitoring
SAE	Serious adverse event
SAP	Statistical analysis plan
SD	Standard deviation
SDV	Source data verification
SOC	System organ class
SOP	Standard operating procedures
SPE	Solid phase extraction
TMF	Trial master file
ULDCT	Ultra low dose computerised tomography
V	Volt
VOI	Volume of interest
WBC	White blood cell
WHO	World Health Organisation

4 IMPORTANT MEDICAL PROCEDURES TO BE FOLLOWED BY THE INVESTIGATOR

4.1 Medical emergencies contacts

The Principal Investigator is responsible for ensuring that procedures and expertise are available to handle medical emergencies during the study. A medical emergency usually constitutes a serious adverse event (SAE) and is to be reported as such. Detailed SAE reporting procedures are described in Section 11.4.2.10.

In the case of a medical emergency, the Investigator may contact the Medical Monitor.

Name	Function in the study	Telephone number and e-mail
[redacted]	Medical Monitor	[redacted]

5 INVESTIGATOR AND STUDY ADMINISTRATIVE STRUCTURE

Sponsor
Nerudia Ltd,
Wellington house
Physics road, Speke
Liverpool, L24 9HP
United Kingdom

Sponsor's Project Manager
[redacted]

Clinical conduct
CTC Clinical Trial Consultants AB (CTC)
Uppsala University Hospital,
Entrance 85, 2nd level
SE-751 85 Uppsala, Sweden

Principal Investigator
[redacted]

PET investigation site
PET Centre, Uppsala University Hospital,
Entrance 85, Ground flooor
SE-751 85 Uppsala, Sweden

Head of PET Centre
[redacted]

Clinical Project Manager
[redacted]

Study management
CTC
Dag Hammarskjölds väg 10B
SE-752 37 Uppsala, Sweden

Clinical Research Manager
[redacted]

Biostatistician

[redacted]

Medical Writer

[redacted]

Medical Monitor

[redacted]

[redacted] provided in Section 19.

6 INTRODUCTION

6.1 Background

E-cigarettes have become a popular alternative to cigarette smoking and are garnering significant attention as potentially reduced exposure products and smoking cessation products. E-cigarettes consist of a battery, heating component, and a reservoir (often referred to as a pod, cartridge, or tank) containing tobacco-derived nicotine in a solution composed of glycerine and/or propylene glycol and flavourings. Upon activation, the heating element heats the solution and the consumer inhales the resulting vapour.

E-liquid formulations aim to provide smokers with an experience that would allow for maximal displacement of combustible cigarettes while at the same time not serving as a gateway to other more toxic products for non-smokers. Key elements that contribute to an acceptable initiating experience include the nicotine content in the products as well as the response that consumers have in terms of satisfaction and prevention of symptoms of withdrawal. One commercially successful development in e-liquid formulations is the use of nicotine salts, which reproduce a form of nicotine naturally available in the tobacco leaf and are able to provide higher levels of nicotine delivery.

6.1.1 *my*blu™ e-cigarette system

*my*blu™ is a commercially available, two-piece, closed e-cigarette system comprised of a rechargeable 350 milliampere hour (mAh) battery and a disposable pod. The pods connect directly and contain the mouthpiece, heating element, are pre-filled with e-liquid, and are compatible only with *my*blu™ batteries. During use, a consumer inhales through the mouthpiece and a sensor in the battery detects the change in air pressure which activates the heating element. The e-liquid heats to an aerosol which the consumer inhales.

The battery is charged with a micro-USB charger and produces a typical output of 3.7 Volt (V) (maximum 3.9 V). Under typical usage conditions, the pods would contain 1.5 millilitre (mL) of e-liquid which lasts approximately 200 puffs, depending on individual use behaviours. Each e-liquid contains a mixture of glycerine, propylene glycol, nicotine freebase, and a proprietary blend of flavours.

6.1.2 *Radiochemistry*

S-[Methyl-^{11}C]nicotine is synthesised according to Figure 6.1-1.

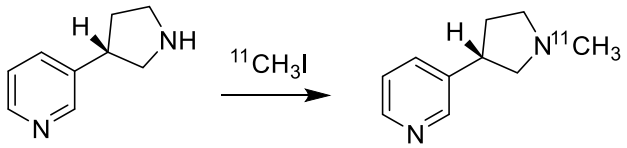

Figure 6.1-1 Synthetisation of *S*-[Methyl-^{11}C]nicotine

[^{11}C]nicotine is purified from the crude product by separation on high-pressure liquid chromatography (HPLC) and obtained in ammonium formate (50 mM pH 3.5) and acetonitrile. The volume is reduced by evaporation to remove most of the acetonitrile and then the product solution is made alkaline by addition of Na_2CO_3 and passed through a C18 solid phase extraction column (SPE). The C18 SPE is washed with sterile water followed by flushing with nitrogen gas to remove ammonium formate and acetonitrile and most of the excess of water. The retained [^{11}C]nicotine is eluted using the Nerudia nicotine solution to be

used in the inhalation device. The amount of radioactivity in the eluate is measured and the inhalation device is filled with appropriate volume corresponding to delivery of 25 megabecquerel (MBq) by a 3-5 second inhalation. A volume of about 10-24 µL is delivered to the subject. The volume administered is dependent on the yield of the ^{11}C-labelling, that may vary between batches.

The inhalation solution is quality controlled by:

- Measurement of radioactivity
- Determination of radiochemical purity
- Determination of concentration of residual acetonitrile, to assure the level is below the IHC recommendations

6.2 Study rationale

This study seeks to investigate the differences in airway deposition of nicotine from e-liquid vapour, between different e-liquid formulations. Two formulations are included in the study, one which uses freebase nicotine and one that uses nicotine salts (nicotine lactate). Both freebase nicotine and nicotine salts (nicotine lactate) are available in commercial products and previous clinical research carried out by Imperial Brands and by others in the industry has demonstrated differences in the pharmacokinetics (PK) between freebase and nicotine salts. This study aims to identify if the differences in PK responses between the formulations are linked to different sites of absorption of the nicotine. By radiolabelling and imaging, we aim to identify where the nicotine is within the respiratory tract which may give insight into the site of nicotine absorption.

In addition to differences in PK, the formulations have different chemical properties. Nicotine has greater volatility in the freebase form compared to nicotine salts which has an impact on the sensorial effect when using the product, with greater throat irritation with freebase compared to nicotine salt formulations. This, in combination with literature on Nicorette Inhalator which demonstrated that gas phase nicotine mostly did not enter the lung, gives an indication that nicotine in the different formulations may reach different areas of the respiratory tract.

6.3 Risk/benefit assessment

It may be considered problematic to expose research subjects to a nicotine delivery product. However, all research subjects are required to be daily cigarette users, so the participants are well acquainted with, and used to, the effects of nicotine.

The investigational product (IP) *my*blu™ is currently commercially available on multiple markets. The amount of nicotine inhaled from a single puff of the IP is lower than in conventional tobacco-based cigarettes. This suggests that adverse effects from the nicotine exposure from the IP are less likely to occur among the research subjects. So far, no adverse effects have been reported associated with the use of the IP apart from known effects likely to be related to the nicotine exposure (such as salivation, nausea, and dyspepsia).

Subjects below the age of 50 years are excluded from the study due to the radiation exposure. In addition, subjects with respiratory diseases or ongoing respiratory infections will be excluded from participation due to potential risks related to IP inhalation.

Risk associated with the e-cigarettes
A risk assessment of the e-cigarette has been conducted as part of design control requirements. The identified hazards were mostly minor or moderate in severity and were mostly expected to occur only rarely. Potential hazards with a rating of "severe" or above included exposure to formaldehyde, nitrosamines or other contaminants, incorrect charging leading to some e-cigarettes overheating, and microbial infection from sharing devices. Chemical analysis of the condensate has confirmed very low levels of contaminants, and in the present study the e-cigarette will be used under supervision in a controlled environment. Therefore, the likelihood of any of these potential identified hazards occurring is considered to be extremely low.

Risk associated with the arterial catheter
There is a small risk for pain upon insertion, and also a few days after removal, of the arterial catheter. There is also a minor risk for numbness as well as impaired blood flow. Sufficient circulation is tested for with modified Allen test at screening. To minimise the risks with arterial catheters, the insertion is done by an anaesthesiologist, under hygienic conditions. Upon removal of the catheter, compression over the artery will be held for approximately 10 minutes, after which a compressing bandage is applied.

Risk associated with the radiation exposure
Radioactively labelled nicotine will be given to the study subjects. The amount of radioactivity expected in the device is estimated to be about 200 MBq at the time of administration. Approximately up to 25 MBq is administered to the subject by two 3-5 second inhalations, as 1-2 consecutive puffs withdrawn from the device for each tracer administration. The effective dose of 50 MBq (2 inhalation administrations) of [^{11}C]nicotine is not likely to exceed 0.2 millisievert (mSv).

The effective dose in radiation protection and radiology is a measure of the cancer risk to a whole organism due to ionising radiation delivered non-uniformly to part(s) of its body. It takes into account both the type of radiation and the nature of each organ being irradiated. Additionally, the Ultra Low Dose Computed Tomography (ULDCT) used for attenuation correction and for anatomical orientation, will give a radiation dose of approximately 0.28 mSv for the entire study. For subjects participating in Part B (Brain scan only) the dose from ULDCT is 0.05 mSv per investigation.

The total radiation dose to the subjects participating in this study with two positron emission tomography/computerised tomography (PET/CT) investigations and two inhalations of [^{11}C]-labelled nicotine will thus be less than 1.0 mSv (0.5 mSv for part A and 0.3 mSv for part B). This dose falls within Category I that allows up to 1 mSv for subjects with an age above 50 years. This dose limit relies on the guideline: "European commission Radiation protection 99, Guidance on medical exposures in medical and biomedical research. 1998 Directorate-General Environment, Nuclear Safety and Civil protection".

To our knowledge, there are no publications reporting effective dose due to [^{11}C]nicotine inhalation or intravenous injection. The Swedish Radiation Safety Authority states an effective dose of 0.004 mSv/MBq for intravenous injection of neuroreceptor ligands, compared to 0.003 mSv/MBq for ^{11}CO inhalation. Homogeneous distribution of a [^{11}C]-labelled tracer results in 0.003 mSv/MBq as well. Based on this data, the effective dose of 50 MBq [^{11}C]nicotine inhalation is not likely to exceed 0.004 mSv/MBq, or 0.2 mSv.

Bergström et al, [1] reported 45% of administered nicotine vapour in the oral cavity at end of inhalation, and 8% at 45 minutes past inhalation. Assuming that 50% of the inhaled activity (25 MBq) remains on the mucosa of the oral cavity, with an effective half life of ten minutes,

and that all beta radiation is absorbed in the top 1 mm layer of the mucosa, the dose to oral cavity mucosa is estimated to be around 60 milligray (mGy). These absorbed doses are far below levels resulting in any risks of deterministic effects (e.g., 6 Gy for skin rash), even though mucosa is probably more radiation sensitive than regular skin. In another PET study reporting inhalation of [^{11}C]nicotine [2], far larger amounts of radioactivity were inhaled (170 MBq per inhalation) without any adverse effect.

Risk associated with the magnetic resonance imaging (MRI)
Subjects who have any condition that is contraindicated with the MRI examination will be excluded for PART B.

Incidental findings during the MRI investigation will be evaluated and documented by a radiologist at the imaging site according to local practice. A clinical statement based on the evaluation will be provided to the Principal Investigator, who will inform the subject about the findings and handle the subject's medical care as per standard medical/clinical judgement.

In order to protect subjects and equipment during the MRI examination all personal and medical metallic and magnetic items (including credit cards etc.) have to be left outside the MRI room. In extensive research to evaluate whether the magnetic fields and radio waves used during an MRI scan pose a risk to the human body, no evidence for any risks have been identified. MRI is thus considered one of the safest medical imaging procedures currently available.

General risks
Besides the risks related to the insertion of arterial catheters, MRI and radiation as described above, there may also be risks related to the medical devices used in the study e.g. clinical laboratory sampling materials. However, these are devices that are used in routine medical care and the risk associated with their use is considered low and ethically justifiable. Study specific evaluations and sampling procedures, like blood-pressure measurements using a blood pressure cuff, may cause transient discomfort but the risk is deemed to be low and ethically justifiable.

Subjects will remain in the research clinic for at least 2 hours after the administration of the IP and will be closely monitored by medical staff. Visits at the clinic may be prolonged in case the Investigator finds it medically warranted for safety reasons.

Overdosing is not likely to occur since all IP will be administered by site personnel under medical surveillance. In cases of accidental overdose, standard supportive measures should be adopted as required. For further information regarding overdosing, refer to Section 11.4.2.13.

The Principal Investigator at the research clinic will ascertain that adequate facilities and procedures are available to handle unexpected safety situations should they occur during the study.

The potential adverse effects of the study procedures, which are likely to be minor and/or clinically insignificant, are from a research ethics perspective counterbalanced by the potential positive effects of the novel nicotine delivery system as a reduced toxicity alternative to conventional cigarettes. As the nicotine disposition and deposition profile of a product is likely to be central to its acceptability among current tobacco users, it is reasonable to conduct formal clinical studies to assess these features in more detail.

7 STUDY OBJECTIVES AND ENDPOINTS

7.1 Primary objectives (PART A only)

The primary objectives are:

- To measure the amount of nicotine deposited in the lungs and the oral cavity during 40 minutes after administration of ^{11}C-labelled nicotine via the *my*blu™ e-cigarette system using PET

7.1.1 *Primary endpoint (PART A only)*

The primary endpoints are:

- Dynamic PET Digital Imaging and Communications in Medicine (DICOM) images over the lungs and oral cavity 0-40 minutes after administration
- Radioactivity vs. time profiles for the lungs and oral cavity 0-40 minutes after administration

7.2 Secondary objectives (PART A only)

The secondary objectives are:

- To describe the single-dose pharmacokinetics of ^{11}C-labelled nicotine, given via the *my*blu™ e-cigarette system, by measuring the radioactivity in arterial blood in discrete samples up to 30 minutes following administration
- To evaluate safety and tolerability of ^{11}C-labelled nicotine inhaled via the *my*blu™ e-cigarette system

7.2.1 *Secondary endpoints (PART A only)*

- Radioactivity vs. time profiles for arterial blood at 2, 4,6, 8, 10, 15, 20 and 30 minutes after administration of [^{11}C]nicotine inhalation
- Frequency, intensity and seriousness of adverse events (AEs)
- Clinically significant changes in vital signs

7.3 Exploratory objectives (PART B only)

The exploratory objective for PART B is:

- To measure the amount of nicotine deposited in the brain during 30 minutes after administration of ^{11}C-labelled nicotine via the myblu™ e-cigarette system using PET
- To describe the single-dose pharmacokinetics of ^{11}C-labelled nicotine, given via the *my*blu™ e-cigarette system, by measuring the radioactivity in arterial blood in discrete samples up to 30 minutes following administration
- To evaluate safety and tolerability of ^{11}C-labelled nicotine inhaled via the *my*blu™ e-cigarette system

7.3.1 *Exploratory endpoints (PART B only)*

- Dynamic PET DICOM images over the brain 0-30 minutes after administration
- Radioactivity vs. time profiles for arterial blood at 2, 4,6, 8, 10, 15, 20 and 30 minutes after administration of [^{11}C]nicotine inhalation
- Frequency, intensity and seriousness of AEs
- Clinically significant changes in vital signs

8 STUDY DESIGN

8.1 Overall study design and schedule of events

This is a single centre, exploratory PET-study of deposition, disposition and brain uptake of [^{11}C]nicotine when given to smokers as two different formulations via the *my*blu™ e-cigarette system.

The study is divided in two parts:

PART A: PET 1 and PET 2 (including 5 subjects/formulation, total 10 subjects)

PART B: PET 3 (including 2 subjects/formulation + 3 optional subjects, total 4-7 subjects)

An overview of the study design is shown in Figure 8.1-1.

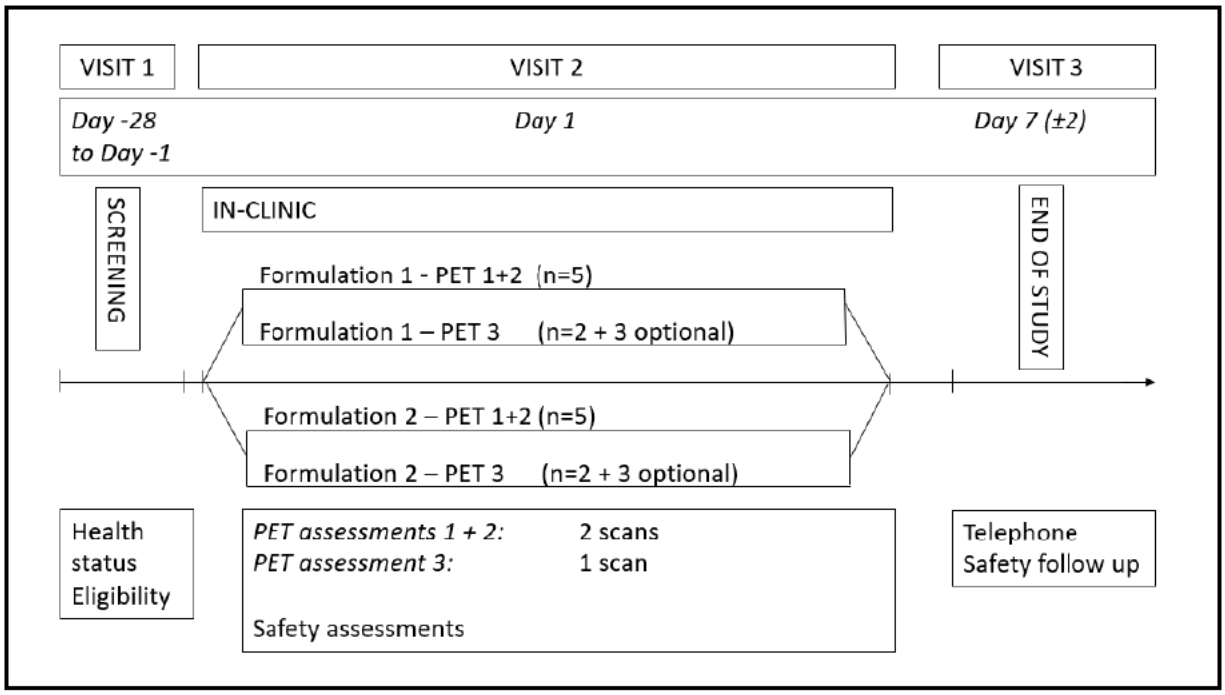

Figure 8.1-1 Overview of the study design

8.1.1 *PART A: PET 1 and PET 2*

In PART A of the study, two different formulations of [^{11}C]nicotine will each be given to 5 subjects. The subjects will have two PET-assessments each (PET 1 and PET 2) after inhalation of [^{11}C]nicotine via the *my*blu™ during the same treatment day. PART A will be completed for 5 subjects on each formulation before initiation of PART B.

The IP will be given as inhalation ("puffs") via the *my*blu™ e-cigarette system to obtain a tracer dose of up to 25 MBq/PET-session.

Subjects will come for 2 visits to the clinic and have 1 End-of Study follow-up telephone call.

Screening (Visit 1) will take place from Day -28 to Day -1 and will include an eligibility check, review of health status and inhalation training, see Table 8.1-1 for details.

At Visit 2, subjects will arrive to the clinic in the morning of Day 1 and will remain at the clinic until the afternoon. Day 1 will contain pre-PET safety assessment, preparation for the

PET assessments (including insertion of an arterial catheter), two PET assessment sessions with at least 2 hours between the sessions and safety follow-up (including removal of arterial catheter) after the second PET assessment.

The subjects will have breakfast (optional) at the research clinic before the first PET-session. Lunch will be served at the research clinic between the two PET-sessions.

The subjects will be carefully monitored by clinical staff during and after the PET-sessions. There is immediate access to equipment and qualified staff in case of emergency.

A final End-of-Study phone call (Visit 3) will take place on Day 7 (±2) or after early withdrawal to follow up on safety (AEs and concomitant medications).

Each subject is expected to participate in the study for approximately 7 days from the day of PET-sessions (Day 1, Visit 2) to the End-of Study Visit. The Screening Visit will be performed within 28 days prior to PET-scanning (Day -28 to Day -1).

The schedule of events is shown in Table 8.1-1.

Study assessments are described in Section 11.

Table 8.1-1 Schedule of events PART A

Visit	**Refer to CSP section:**	**Visit 1**	**Visit 2[0]**				**Visit 3**
		Screening	**Day 1**				**End-of-study**
							Phone call
Assessment/ Approximate timepoints		Day -28 to Day -1	Admission 08:00	10:00	12:00	14:00	16:00	Day 7 (±2)[2]
Informed Consent	14.3	X						
Inclusion/exclusion criteria	9.4, 9.5	X	X[3]					
Demographics	11.2	X						
Medical/surgical history	11.2.5	X						
HIV, hepatitis B and C	11.2.7	X						
Alcohol breath test	11.2.9	X	X					
Urine Drug Screen	11.2.8	X	X					
Weight/height (Body Mass Index [BMI])	11.2.4	X						
Physical Examination	11.2.12	X						
Clinical Laboratory Profile	11.2.11	X						
Vital Signs	11.4.3	X					X	
12-lead ECG	11.2.13	X						
Inhalation training	11.3.1	X	(X)					
Modified Allen test	11.3.2	X	X					
Insertion of arterial catheter	11.3.2		X					
PET assessment[4]	11.2.11			X		X		
Sampling arterial blood for radioactivity assessment[4]	11.2.11			X		X		
IP administration[4]	10.5			X		X		
Meals at the research clinic	9.6		X		X			
Removal of arterial catheter	11.3.2						X	
Discharge from research clinic							X	
Baseline symptoms	11.2.10	X	X					
Adverse events	11.4.2			---------- X ----------				
Prior and concomitant medications	11.2.6	---------- X ----------						

CSP= Clinical Study Protocol, HIV= Human Immunodeficiency Virus, ECG= Electrocardiogram

1. Timepoints given for Visit 2 are approximate and may be adjusted over the day if needed for logistical reasons in agreement between the research clinic and the PET-Centre.
2. Or after early withdrawal.
3. Confirmation of eligibility.
4. Activities performed at the PET-Centre

8.1.2 ***PART B: PET 3***

In PART B of the study, two different formulations of [^{11}C]nicotine will each be given to 2 subjects. The subjects will have one PET-assessments each (PET 3) after two inhalations of [^{11}C]nicotine via the *my*blu™.

PART B will be completed for 2 subjects on each formulation before a decision will be taken if 3 additional subjects should be added to PART B. The decision is based on whether the results from the first scans show significant, quantifiable uptake of radioactivity in the brain. If 3 additional subjects are included, they will be treated with the formulation that showed the best uptake profile.

The IP will be given as inhalation ("puffs") via the *my*blu™ e-cigarette system to obtain a tracer dose of up to 50 MBq.

Subjects will come for 2 visits to the clinic, 1 MRI assessment visit (if not on the PET assessment day) and have 1 End-of Study follow-up telephone call (Visit 3).

Screening (Visit 1) will take place from Day -28 to Day -1 and will include an eligibility check, review of health status and inhalation training, see Table 8.1-2 for details.

At Visit 2, subjects will arrive to the clinic in the morning of Day 1 and will remain at the clinic until the afternoon. Day 1 will contain pre-PET safety assessment, preparation for the PET assessments (including insertion of an arterial catheter), one PET assessment session and safety follow-up (including removal of arterial catheter) after the PET assessment.

The subjects will have breakfast (optional) at the research clinic before the PET-session. Lunch (optional) will be served at the research clinic after the PET session.

The subjects will be carefully monitored by clinical staff during and after the PET-session. There is immediate access to equipment and qualified staff in case of emergency.

A final End-of-Study phone call (Visit 3) will take place on Day 7 (±2) or after early withdrawal to follow up on safety (AEs and concomitant medications).

During the study period, the subjects will also have an MRI-scan. The MRI can be performed at any suitable timepoint during the study after the screening visit until the End-of-Study-visit.

Each subject is expected to participate in the study for approximately 7 days from the day of PET-session (Day 1, Visit 2) to the End-of Study Visit. The Screening Visit will be performed within 28 days prior to PET-scanning (Day -28 to Day -1).

The schedule of events is shown in Table 8.1-2.

Study assessments are described in Section 11.

Table 8.1-2 Schedule of events PART B

Visit	**Refer to CSP section:**	**Visit 1**	**Visit 2[1]**			**Visit 3**
		Screening	**Day 1**			**End-of-study**
						Phone call
Assessment/ Approximate timepoints		Day -28 to Day -1	Admission 08:00	10:00	12:00	Day 7 (±2)[2]
Informed Consent	14.3	X				
Inclusion/exclusion criteria	9.4, 9.5	X	X[3]			
Demographics	11.2	X				
Medical/surgical history	11.2.5	X				
HIV, hepatitis B and C	11.2.7	X				
Alcohol breath test	11.2.9	X	X			
Urine Drug Screen	11.2.8	X	X			
Weight/height (BMI)	11.2.4	X				
Physical Examination	11.2.12	X				
Clinical Laboratory Profile	11.2.11	X				
Vital Signs	11.4.3	X			X	
12-lead ECG	11.2.13	X				
Inhalation training	11.3.1	X	(X)			
MRI[4]	11.3.5.1	---------- X----------				
Modified Allen test	11.3.2	X	X			
Insertion of arterial catheter	11.3.2		X			
PET assessment[5]	11.2.11			X		
Sampling arterial blood for radioactivity assessment[5]	11.2.11			X		
IP administration[5]	10.5			X		
Meals at the research clinic	9.6		X		(X)	
Removal of arterial catheter	11.3.2				X	
Discharge from research clinic					X	
Baseline symptoms	11.2.10	X	X			
Adverse events	11.4.2			---------- X ----------		
Prior and concomitant medications	11.2.6	---------- X ----------				

1. Timepoints given for Visit 2 are approximate and may be adjusted over the day if needed for logistical reasons in agreement between the research clinic and the PET-Centre.
2. Or after early withdrawal.
3. Confirmation of eligibility
4. An MRI can be performed at any suitable timepoint after the subject's inclusion in the study at the screening visit until the End-of-Study-visit.
5. Activities performed at the PET-Centre

9 STUDY POPULATION

Prospective approval of protocol deviations to eligibility criteria, also known as protocol waivers or exemptions, is not permitted.

9.1 Recruitment

The subjects will be recruited from CTC's database of healthy volunteers and patients and from advertising in media (including social media).

Subjects may not participate in more than one part of the study.

9.2 Screening and enrolment log

Investigators must keep a record of all screened subjects even if they were not subsequently included in the study. This information is necessary to verify that subjects were selected without bias. The reason for screen failure should be stated for all subjects screened but not included. The reason for withdrawal should be stated for all subjects included but not completed.

A screening number will be allocated to each subject in connection to the informed consent process at the Screening visit. The screening number is generated automatically in the electronic case report Form (eCRF). The screening number will allow identification of subjects irrespective of their possible eligibility for the study.

Subjects included will be assigned a subject number (101, 102, 103 etc. for PART A and 201, 202, 203 etc. for PART B). The first digit will correspond to the study part, and the 2nd and the 3rd digits correspond to the subject number.

If a subject cannot receive the planned dose of IP within 28 days after screening (*i.e.*, the time interval between signing informed consent until dose administration) the subject should be rescreened before proceeding in the study.

9.3 Number of subjects

Approximately 25 subjects will be screened to achieve a total of 14-17 included and evaluated subjects.

For replacements of subjects who discontinue from the study, see Section 9.8.

9.4 Inclusion criteria

For inclusion in the study, subjects must fulfil the following criteria:

1. Willing and able to give written informed consent for participation in the study.
2. Male subject or female subject of non-child bearing potential, aged 50-65 years inclusive.
   Women of non-childbearing potential are defined as pre-menopausal females who are sterilised (tubal ligation or permanent bilateral occlusion of fallopian tubes); or post-menopausal defined as 12 months of amenorrhea (in questionable cases a blood sample

with simultaneous detection of follicle stimulating hormone [FSH] 25-140 IE/L and oestradiol <183 pmol/l is confirmatory).

3. BMI ≥ 18.0 and ≤ 30.0 kg/m^2.
4. Habitual daily cigarette smoker.

9.5 Exclusion criteria

Subjects must not enter the study if any of the following exclusion criteria are fulfilled:

1. History of any clinically significant disease or disorder which, in the opinion of the Investigator, may either put the subject at risk because of participation in the study, or influence the results or the subject's ability to participate in the study.
2. Any clinically significant illness, medical/surgical procedure or trauma within 4 weeks of the (first) administration of IP that would influence the results or the subject's ability to participate in the study, as judged by the Investigator.
3. Any clinically significant disease affecting the respiratory tract that would influence the results of the study or the subject's ability to participate in the study, as judged by the Investigator.
4. Malignancy within the past 5 years with the exception of in situ removal of basal cell carcinoma.
5. Any planned major surgery within the duration of the study.
6. Any positive result on screening for serum hepatitis B surface antigen, hepatitis C antibodies and HIV.
7. Untreated hypertension and/or, after 10 minutes supine rest at the time of screening, any vital signs values outside the following ranges:
   - Systolic blood pressure <90 or >160 mmHg, or
   - Diastolic blood pressure <50 or >100 mmHg, or
   - Pulse <40 or >90 bpm
8. Clinically significant abnormalities in the resting ECG at the time of screening that would influence the results of the study or the subject's ability to participate in the study, as judged by the Investigator.
9. Previous participation in a PET-study or other nuclide medical study.
10. Risk of occupational exposure to significant ionising radiation.
11. Previous participation in any clinical procedures involving significant exposure to radiation (exceptions are dental X-rays and common X-rays of chest or extremities).
12. Negative results of the modified Allen test on both arms at the time of screening or pre-arterial catheter insertion at Visit 2.
13. Current ongoing upper respiratory tract infection with e.g. coughing at the day of the PET-assessment, as judged by the Investigator.

14. Regular use of any prescribed or non-prescribed medication for the treatment of respiratory tract disorders within 2 weeks prior to the (first) administration of IP, at the discretion of the Investigator.
15. Regular use of any anticoagulantia medication within 2 weeks prior to the (first) administration of IP, at the discretion of the Investigator.
16. Known allergy towards local anaesthetics of the type used in connection with arterial catheter insertion.
17. Planned treatment or treatment with investigational drugs within 3 months prior to Visit 2.
18. Positive screen for drugs of abuse or alcohol at screening or on admission to the research unit on Visit 2.
19. History of alcohol abuse or excessive intake of alcohol, as judged by the Investigator.
20. Presence or history of drug abuse and/or anabolic steroids, as judged by the Investigator.
21. Investigator considers the subject unlikely to comply with study procedures, restrictions and requirements.
22. *For PART B only:* Conditions contraindicating MRI, including, but not limited to, claustrophobia, metallic implants or internal electrical devices (e.g., cochlear implant, nerve stimulator, gastric pacemaker, bladder stimulator, pacemaker, defibrillator, artificial valves in heart, aneurysm clips, etc.), and permanent makeup or tattoos which in the Investigator's opinion might jeopardise the subject's safety or interfere with the imaging measurements. Subjects with metal fragments in the head or body that would present a risk during the MRI scanning procedure, or subjects who have worked with ferrous metals either as a vocation or hobby (for example, as a sheet metal worker, welder or machinist) in such a way that might have led to unknown indwelling metal fragments that could cause injury if moved in response to the magnetic field during MRI.

9.6 Restrictions during the study

The subjects must be willing to comply to the following restrictions during the entire study duration *i.e.*, from screening to the end-of-study visit.

9.6.1 *General restrictions*

- Meals and Dietary Restrictions: Meals (breakfast and lunch) will be served at the research clinic during Visit 2. Lunch will be served between the two PET-sessions (for subjects participating in PART A). Water is allowed ad libitum at the clinic except during PET-sessions.
- Nicotine: Smoking or use of nicotine-containing products, except the IP, is not allowed during the study from midnight the day before the PET assessment until the subject leaves the clinic after the (last) PET assessment.
- Alcohol: Consumption of alcohol is not allowed within 48 hours prior to or during Visit 2.
- Coffee: Consumption of up to 5 cups of coffee will be allowed during Visit 2.

- Xanthine or taurine containing products/beverages: Energy drinks (e.g. Red bull) are not allowed during Visit 2.
- Blood donation: The subjects must not donate blood or plasma during the study until one month after Visit 2.
- Participation in other clinical studies: Study subjects are not allowed to participate in any other interventional clinical study during the study period.

9.6.2 *Prior and concomitant therapy*

Prohibited medication

- Regular use of any prescribed or non-prescribed medication for the treatment of respiratory tract disorders within 2 weeks prior to the (first) administration of IP, at the discretion of the Investigator.
- Anticoagulantia.

Allowed medication

- Medications considered necessary for treatment of chronic/stable conditions that are part of the subject's medical history, as judged by the Investigator.
- Other medications considered necessary for the subject´s safety and wellbeing may be given at the discretion of the Investigator during the study period.

9.7 Screen failures

Screen failures are defined as subjects who consent to participate in the clinical study but are not subsequently included in the study. A minimal set of screen failure information is required to ensure transparent reporting of screen failure subjects. Minimal information includes documentation of signed and dated informed consent form (ICF) and reason(s) for screening failure.

Re-screening can be performed once if any of the following were reasons for screening failure or non-randomisation (as judged by the Investigator):

- Practical reasons.
- Non-significant medical conditions (e.g. influenza, nasopharyngitis).

For subjects who are re-screened, a new screening number will be assigned and a new, signed ICF will be collected.

9.8 Subject withdrawal

9.8.1 *General withdrawal criteria*

Subjects are free to discontinue their participation in the study at any time and for whatever reason without affecting their right to an appropriate follow-up investigation or their future care. If possible, the reason for withdrawal of consent should be documented.

Subjects may be discontinued from the study at any time at the discretion of the Investigator.

Reasons for discontinuation include:

- Subject decision
- Severe non-compliance to study protocol procedures, as judged by the Investigator and/or Sponsor
- Subject is lost to follow-up.
- Significant AEs posing a risk for the subject, as judged by the Investigator and/or Sponsor
- Withdrawal of informed consent to the use of biological samples
- Pregnancy
- Death
- Meeting of an exclusion criterion during the study, which, in the opinion of the Investigator, may pose a risk for the subject
- Use of prohibited medication

9.8.2 *Procedures for discontinuation of a subject from the study*

A subject who prematurely discontinues participation in the study will always be asked about the reason(s) for discontinuation and the presence of any AEs. If a subject withdraws consent, the Investigator must ask the subject if he/she is willing, as soon as possible, to be assessed according to the procedures scheduled for the end-of-study visit. Any ongoing AEs will be followed as described in Section 11.4.2.11.

The primary reason for discontinuation/early withdrawal must be specified in the eCRF and final drug accountability must be performed.

9.8.3 *Subject replacement*

Subjects who are prematurely withdrawn from the study before completing the intended PET-assessments may be replaced.

9.9 Randomisation

Not applicable.

9.10 Blinding

This is an open-label study, i.e. the Investigator, study staff and subjects will know the type of IP to be received.

10 TREATMENTS

10.1 Identity of investigational products

The IPs will be supplied as the *my*blu™ e-cigarette system with e-liquid pods:

The *my*blu™ e-cigarette will be supplied together with a USB charger.

E-liquid pods will be supplied as:

- Pods for training purposes, containing 2.5% freebase nicotine formulation (no flavour) corresponding to Formulation 1.
- Pods for training purposes, containing 2.5% nicotine salt formulation (no flavour) corresponding to Formulation 2.
- Empty pods (for filling at the PET Centre with radiolabelled Formulation 1 and Formulation 2)

Bulk liquid Formulation 1 and Bulk liquid Formulation 2, will be supplied, for radiolabelling and filling of empty e-liquid pods at the PET Centre.

10.2 Manufacturing, packaging and labelling

The IP is manufactured, packed and labelled by Nerudia Ltd, Liverpool, United Kingdom.

Labelling of e-liquid pods filled with radioactive e-liquid formulation will be labelled individually by the PET Centre before each administration to an individual study subject.

10.3 Conditions for storage

The IP will be stored at room temperature away from direct sunlight and heat.

Temperature logs will be kept for the area where the IP is stored. The temperature should be noted on a daily basis (working days only unless automatic temperature readings are available).

10.4 Preparation and accountability

Preparation of the inhalation device (preparation of radiolabelled formulations and filling of e liquid pods) before each PET-sessions will be done by trained personnel at the PET Centre.

CTC/PET Centre will maintain a Storage and Accountability Log detailing the dates and quantities of study medication received, prepared for and used by each subject and IP returned or destroyed at the end of the study. Any discrepancies between prepared and returned IP must be explained and documented. Products deliberately and/or accidentally destroyed by the site or the subject must be accounted for.

10.5 Treatment administration

Instructions on the proper use of the *my*blu™ products will be provided prior to use. Product use in the study sessions will consist of 1-2 puffs of each 3-5 seconds, followed by an inhalation of the vapour into the lungs.

Inhalation training of study subjects, see 11.3.1.

10.6 Continuation of treatment with Investigational Product

This is an exploratory study in volunteers who will have no medical benefit from the treatment and thus there will be no treatment after end of study participation.

10.7 Treatment compliance

All IP will be administered at the research clinic under supervision of trained study personnel to ensure compliance.

10.8 Return and destruction of investigational products

Any unused study product and all empty containers will be destructed at the site upon confirmation from the Sponsor. The Monitor will perform final IP accountability reconciliation at the study end to verify that all unused IP is adequately destroyed and documented.

11 STUDY ASSESSMENTS

The study assessments are described in the sections below and the timing of these assessments are detailed in the schedule of events (PART A: Table 8.1-1 and PART B: Table 8.1-2)

11.1 Recording of data

The Principal Investigator will provide the Sponsor with all data produced during the study from the scheduled study assessments. He/she ensures the accuracy, completeness, legibility, and timeliness of the data reported to Sponsor in the eCRF and in all required reports.

11.2 Demographics and other baseline characteristics

11.2.1 *Informed consent*

Signed informed consent must be obtained before any screening procedures are initiated. The informed consent procedure is further described in Section 14.3.

11.2.2 *Eligibility criteria*

Eligibility criteria should be checked during screening and verified before IP administration. The criteria are specified in Sections 9.4 and 9.5.

11.2.3 *Demographic information*

The following demographic data will be recorded: gender, age, ethnicity and race.

11.2.4 *Weight and height*

Weight and height will be measured without shoes. BMI will be calculated with one decimal from the height and weight recorded.

11.2.5 *Medical/surgical history*

Medical/surgical history will be obtained by subject interview in order to verify that the eligibility criteria are met.

The medical/surgical history should include all relevant diseases and surgeries within 2 months prior to screening as judged by the Investigator.

11.2.6 *Prior and concomitant medication*

Prior medications taken within 2 weeks will be obtained by subject interview in order to verify that the eligibility criteria are met (see also Section 9.6.2).

Medications are classified as prior if the stop date was before or on the day of the first dose administration (pre-dose) and as concomitant if ongoing on the day of the first dose administration, stopped after the first dose administration or started after the first dose administration. To distinguish between prior and concomitant medications on Day 1 in each part (i.e. the first IP administration), the start time of any newly introduced medication or the stop time of any previously ongoing medication must be recorded in the eCRF.

Any use of concomitant medication from screening until the last end-of-study visit must be documented appropriately in the subject's eCRF. Relevant information (*i.e.* name of medication, dose, unit, frequency, start and stop dates, reason for use) must be recorded. All changes in medication should be noted in the eCRF.

11.2.7 *HIV and Hepatitis B/C*

Subjects will be tested for HIV, hepatitis B surface antigen and hepatitis C antibodies prior to inclusion into the study. Any positive result will exclude the subject from participating in the study.

11.2.8 *Urine drug screen*

Urine will be screened for drugs of abuse at timepoints outlined in the schedule of events (Table 8.1-1 and Table 8.1-2) using the Alere™ Drug Screen Test Panel.

11.2.9 *Alcohol breath test*

An alcohol breath test will be performed at timepoints outlined in the schedule of events (Table 8.1-1 and Table 8.1-2).

11.2.10 *Baseline symptoms*

A baseline symptom is defined as an event that occurs between the subject's signing of the ICF until the insertion of the arterial catheter (i.e. an event that occurs during the screening

period). Such events are not AEs and will be recorded as baseline symptoms in the Medical History Log in the eCRF.

11.2.11 *Clinical laboratory profile*

Blood samples for analysis of clinical chemistry and haematology will be collected through venepuncture or an indwelling venous catheter and sent to the certified clinical chemistry laboratory at Uppsala University Hospital and analysed by routine analytical methods.

The clinical laboratory parameters are defined in Table 11.2-1 and will be assessed at screening.

Abnormal values assessed by the Investigator as clinically significant will be reported as baseline symptoms. If an abnormal value is associated with corresponding clinical signs or symptoms, the sign/symptom should be reported in the Medical History Log in the eCRF.

Table 11.2-1 Clinical laboratory parameters

Category	**Parameter**
Clinical chemistry	Alanine aminotransferase (ALT)
	Aspartate aminotransferase (AST)
	Creatinine
	Glucose
Haematology	Haematocrit
	Haemoglobin (Hb)
	Platelet count
	Red blood cell (RBC) count
	White blood cell (WBC) count
FSH-test (at screening, postmenopausal females only in questionable cases)	FSH
	Estradiol

11.2.12 *Physical examination*

A complete physical examination will include assessments of the head, eyes, ears, nose, throat, skin, thyroid, neurological, lungs, cardiovascular, abdomen (liver and spleen), lymph nodes and extremities.

Physical examination will be judged as normal, abnormal, not clinically significant or abnormal, clinically significant. The assessment will be recorded in the eCRF.

11.2.13 *Resting 12-lead ECG*

Single 12-lead ECG will be recorded in supine position after 10 minutes of rest using an ECG machine. HR and PR, QRS, QT and QTcF intervals will be recorded.

ECGs will be reviewed and interpreted on-site by the Investigator.

Any abnormalities will be specified and documented as clinically significant or not clinically significant.

11.3 Assessments related to PET-endpoints

11.3.1 *Inhalation training*

At the end of the screening visit subjects, who consented to study participation and are found eligible (awaiting results of clinical laboratory sampling), will perform inhalation training with the *my*blu™ e-cigarette. Training will be held by study personnel at the research site.

Inhalation training will be performed on dummy (nicotine free) devices and once on a regular *my*blu™ e-cigarette (containing nicotine corresponding to the formulations used during PET). Training on a nicotine-containing device is considered necessary for the subjects for them to be used to the experience when inhaling in the PET-camera.

Inhalation training on dummy devices (nicotine free) may be repeated in the morning of Visit 2 (before PET), if considered necessary by the study personnel or the subject.

11.3.2 *Insertion/removal of arterial catheter*

After a positive modified Allen test, an intraarterial catheter will be inserted preferably in the arteria radialis of the non-dominant arm if possible, else in another artery chosen by the anaesthesiologist. Local anaesthetics e.g. 0.5 mL of Xylocain® (active substance lidocaine) 10 mg/mL may be applied for the arterial cannulation at the discretion of the anaesthesiologist inserting the cannula.

Positive modified Allen test: If the hand flushes within 5-15 seconds, this indicates that the ulnar artery has good blood flow; this normal flushing of the hand is considered to be a positive test.

Negative modified Allen test: If the hand does not flush within 5-15 seconds, this indicates that ulnar circulation is inadequate or non-existent; in this situation, the radial artery supplying arterial blood to that hand should not be punctured.

The arterial catheter will be removed by trained personnel at the research clinic after the (last) PET session is completed. When the catheter is withdrawn, pressure should be applied to the injection site for approximately 10 minutes until bleeding ceases. The catheter is checked to see that it is intact.

11.3.3 *Assessment of blood radioactivity*

Discrete blood samples will be analysed using an in house developed well counter detector system cross-calibrated with the PET/CT scanner.

Serial discrete arterial blood samples with a volume of approximately 5 mL (2 mL used for each analysis) will be collected in test tubes for the analysis of radioactivity in whole blood withdrawn at specified timepoints (Section 11.3.4 and 11.3.5). The time of sampling will be recorded in the middle of the sampling period by using a foot pedal linked to a computer and the test tubes will be labelled with barcodes for identification. Data will be stored directly in separate files, one for each PET/CT-scan session, including sample weight, number of counts and time of measurement. The data will be corrected for physical decay of ^{11}C to the time for tracer inhalation. The samples will be destructed directly after measurement.

11.3.4 *PET/CT (PART A)*

Administration/inhalation and subject preparation
The subject will be moved from the research site to the PET-Centre approximately 30 minutes before the PET-scan.

The subject is positioned in the PET-CT camera and a ULDCT scan is performed to correct for attenuation of the photons and to outline the anatomy. In supine position the subject inhale from the device, without changing the head or body position. The PET-CT operator takes the inhalation device and the PET-scan begins.

The inhalation device is measured before and after inhalation to determine total amount of [11C]nicotine delivered to the subject. The amount of [^{11}C]nicotine lost when the subject exhales cannot be determined.

PET-protocol
PET-scan 1: The subject inhales (1-2 puffs) for about 3-5 seconds to receive about 10-24 µL solution corresponding to approximately 25 MBq [^{11}C]nicotine. A dynamic 40 min PET scan is performed with the oral cavity/throat and large bronchi in field-of-view (20 cm). Arterial blood is collected as discrete samples of 5 mL each, taken at 2, 4, 6, 8, 10, 15, 20 and 30 minutes to determine radioactivity in whole blood.

PET-scan 2: The subject inhales (1-2 puffs) for about 3-5 seconds to receive about 10-24 µL solution corresponding to approximately 25 MBq [^{11}C]nicotine. A dynamic 40 min PET scan is performed with part of the lung in field-of-view (20 cm). Arterial blood is collected as discrete samples of 5 mL each, taken at 2, 4, 6, 8, 10, 15, 20 and 30 minutes to determine radioactivity in whole blood.
For both lung and oral cavity scans, the dynamic PET data is acquired in list mode, and will initially be reconstructed into 6 x 10s, 3 x 20s, 2 x 30s, 2 x 60s, 2 x 150s and 6 x 300s frames. The scanner will be started 30 seconds before the tracer administration. The data collected 30 seconds before inhalation will not be reconstructed. The registration of data is depending on the resulting images, the frame definition may be optimised, taking the inhalation protocol into account. The static scans over lung and oral cavity will be acquired as single frame scans of 600s.

11.3.5 *PET/MR (PART B)*

PET scan 3: The subject inhales (1-2 puffs) for about 3-5 seconds to receive about 12 µL solution corresponding to approximately 50 MBq [^{11}C]nicotine. A dynamic PET scan is performed over the brain 0-30 min. Arterial blood is collected as discrete samples of 5 mL each, taken at 2, 4, 6, 8, 10, 15, 20 and 30 minutes to determine radioactivity in whole blood.

Optional – extended brain uptake study
If the results from PET-scan 3 show significant, quantifiable, uptake of radioactivity in the brain, the study is extended to include 3 more subjects, to a total of 5 individuals receiving the formulation that shows the best uptake.

For the brain scans, the dynamic PET data is acquired in list mode, and will initially be reconstructed into 6 x 10s, 3 x 20s, 2 x 30s, 2 x 60s, 2 x 150s and 4x 300s frames. The scanner will be started 30 seconds before the tracer administration. The data collected 30s before inhalation will not be reconstructed. The registration of data is depending on the resulting images, the frame definition may be optimised, taking the inhalation protocol into account.

11.3.5.1 MR-scan for anatomical localisation of [^{11}C]nicotine uptake:

All subjects participating in PART B will perform an MRI at any suitable timepoint during the study period (after screening until end-of-study- visit). With MRI, a standard structural T1-weigthed volume is acquired to be used for anatomical localisation of [^{11}C]nicotine uptake in combination with PET scans acquired.

11.4 Assessments related to secondary endpoints

11.4.1 *PET endpoints*

Refer to Section 11.3.

11.4.2 *Adverse events*

The Principal Investigator is responsible for ensuring that all medical staff involved in the study is familiar with the content of this section and the content of the CTC standard operating procedures (SOPs) regarding handling of AEs.

11.4.2.1 Definition of adverse event

An AE is defined as any untoward medical occurrence in a subject administered an IP and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavourable and unintended sign (including clinically significant abnormal values from relevant tests, such as clinical safety laboratory tests, ECGs, vital signs), symptom, or disease temporally associated with the use of an IP, whether or not considered related to the IP.

11.4.2.2 Definition of serious adverse event

An SAE is any AE which:

- results in death
- is life-threatening (this refers to a reaction in which the subject was at risk of death at the time of the reaction; it does not refer to a reaction that hypothetically might had led to death if the reaction was more severe)
- requires in-patient hospitalisation or prolongation of existing hospitalisation
- results in persistent or significant disability/incapacity
- is a congenital anomaly/birth defect
- is an important medical event (IME) (this refers to a reaction that may not be immediately life-threatening or result in death or hospitalisation, but may jeopardise the subject or may require intervention to prevent any of the other outcomes defined above)

Examples of IMEs are intensive treatment in an emergency room for allergic bronchospasm or blood dyscrasias, convulsions that do not result in hospitalisation, development of drug dependency, and drug abuse.

Planned hospitalisations or surgical interventions for a condition that existed before the subject signed the ICF and that did not change in intensity are not SAEs.

If there is any doubt as to whether an AE meets the definition of an SAE, a conservative viewpoint must be taken, and the AE must be reported as an SAE.

11.4.2.3 Time period and frequency for collecting adverse events

All AEs (including SAEs) will be collected from the insertion of the arterial catheter until the end-of-study visit.

Any AE with start date on the day IP administration must be recorded with start time.

At the end-of-study visit, information on new AEs or SAEs, if any, and stop dates for ongoing events must be recorded as applicable.

Investigators are not obligated to actively seek AEs or SAEs after conclusion of the study participation. However, if the Investigator learns of any SAE, including a death, at any time after a participant has been discharged from the study, and he/she considers the event to be reasonably related to the study intervention or study participation, the Investigator must promptly notify the Sponsor.

11.4.2.4 Assessment of intensity

The grading of the intensity of AEs will follow the common terminology criteria for adverse events (CTCAE) v5.0 [3]. Grade refers to the severity of the AE. The CTCAE displays Grades 1 through 5 with unique clinical descriptions of severity for each AE based on this general guideline.

The Investigator must assess the intensity of an AE using the following definitions, and record it on the AE Log in the eCRF:

Grade 1	Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
Grade 2	Moderate; minimal, local or non-invasive intervention indicated; limiting age-appropriate instrumental activities of daily living (ADL)*.
Grade 3	Severe or medically significant but not immediately life-threatening; hospitalisation or prolongation of hospitalisation indicated; disabling; limiting self- care ADL**.
Grade 4	Life-threatening consequences; urgent intervention indicated.
Grade 5	Death related to AE.

**Instrumental ADL refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.*

***Self- care ADL refer to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.*

11.4.2.5 Assessment of causal relationship

The Investigator must assess the causal relationship between an AE and the IP using the definitions below and record it the AE Log of the eCRF:

Probable	The event has a strong temporal relationship to the IP or recurs on re-challenge, and another aetiology is unlikely or significantly less likely.

Possible	The event has a suggestive temporal relationship to the IP, and an alternative aetiology is equally or less likely.
Unlikely	The event has no temporal relationship to the IP or is due to underlying/concurrent illness or effect of another drug (that is, there is no causal relationship between the IP and the event).

An AE is considered causally related to the use of the IP when the causality assessment is probable or possible.

In addition, the Investigator will assess the causal relationship between an AE and the study procedures (e.g. injection site reaction, events in association with arterial catheter insertion, other arterial catheter associated AE).

11.4.2.6 Assessment of outcome

The Investigator must assess the outcome of an AE using the definitions below and record it on the AE Log of the eCRF:

Recovered/resolved	The subject has recovered completely, and no symptoms remain.
Recovering/resolving	The subject's condition is improving, but symptoms still remain.
Recovered/resolved with sequelae	The subject has recovered, but some symptoms remain (for example, the subject had a stroke and is functioning normally but has some motor impairment).
Not recovered/not resolved	The subject's condition has not improved and the symptoms are unchanged (for example, an atrial fibrillation has become chronic).
Fatal	
Unknown	

11.4.2.7 Reporting of action taken with study treatment

The Investigator must report the action taken with study treatment using the definitions below and record it on the AE Log of the eCRF:

Dose increased

Dose not changed

Dose rate reduced

Dose reduced

Drug interrupted

Drug withdrawn

Not applicable

Unknown

11.4.2.8 Collecting adverse events

AEs identified using any of the following methods will be recorded:

- AEs spontaneously reported by the subject

- AEs observed by the Investigator or medical personnel
- AEs elicited based on non-leading questions from the Investigator or medical personnel

11.4.2.9 Recording adverse events

AEs must be recorded in the AE Log of the eCRF. The Investigator must provide information on the AE, preferably with a diagnosis or at least with signs and symptoms; start and stop dates, start and stop time; intensity; causal relationship to IP or study procedures; action taken, and outcome.

If the AE is serious, this must be indicated in the eCRF.

AEs, including out-of-range clinically significant clinical safety laboratory values, must be recorded individually, except when considered manifestations of the same medical condition or disease state; in such cases, they must be recorded under a single diagnosis.

Malfunctions/incidents with the e-cigarette system will be reported to the Sponsor on a separate Product Form.

11.4.2.10 Reporting of serious adverse events

SAE reporting should be performed by the Investigator within 24 hours of awareness via the eCRF. All available information regarding the SAE should be entered in the AE Log for the specific subject. By saving the event as "serious" in the eCRF and once the Investigator has signed-off of the event, an e-mail alert is automatically sent to predefined recipients to highlight that an SAE has been registered. The same information is automatically sent to sae@ctc-ab.se.

The SAE report is reviewed by a designated person at CTC's Pharmacovigilance (CTC PV) department to ensure that the report is valid and correct. For fatal or life-threatening SAEs where important or relevant information is missing, immediate follow-up is undertaken and queries to the site are raised. Investigators or other site personnel should inform CTC PV of any follow-up information on a previously reported SAE immediately but no later than the end of the next business day of when he or she becomes aware of it.

If the SAE report in the eCRF is updated, a new e-mail alert will be sent.

If any additional documentation is required (e.g. autopsy report), CTC PV will request this information from the study site.

In case the eCRF cannot be accessed, the SAE should be reported by manual completion of the paper SAE Form, provided in the Investigator Site File (ISF). The completed, signed and dated paper SAE Form should, within 24 hours, be scanned and e-mailed to:

[redacted]

A copy of the paper SAE form must also be e-mailed to CTC at: sae@ctc-ab.se.

The study site should notify the site Monitor via phone or e-mail about the submission of the SAE report. As soon as the site personnel have access to the eCRF, the SAE should be reported electronically as well.

The Sponsor or delegate will assume responsibility for reporting SAEs to the independent ethics committee (IEC) in accordance with local regulations.

11.4.2.11 Treatment and follow-up of adverse events

Subjects with AEs that occur during the study must be treated according to daily clinical practice at the discretion of the Investigator.

AEs must be followed up until resolution or to the end-of-study visit, whichever comes first. At the end-of-study visit, information on new AEs, if any, and stop dates for previously reported AEs must be recorded (if known). AEs assessed as stable by the Investigator at the end-of-study visit will not have to be followed up until resolution.

It is the responsibility of the Investigator to follow up on all SAEs until the subject has recovered, stabilised, or recovered with sequelae, and to report to the Sponsor all relevant new information using the same procedures and timelines as those for the initial report. Relevant information includes discharge summaries, autopsy reports, and medical consultation.

11.4.2.12 Procedures in case of pregnancy

In case of pregnancy or suspicion of possible pregnancy of any female subjects, the study treatment must be stopped immediately, and the subject discontinued from participation in the study. Pregnancy itself is not regarded as an AE unless there is a suspicion that the IP may have interfered with the effectiveness of the contraceptive medication. However, the outcome of all pregnancies (spontaneous miscarriage, elective termination, normal birth or congenital abnormality) must be followed up and documented even after the subject was discontinued from the study.

All events of congenital abnormalities/birth defects are SAEs. Spontaneous miscarriages should also be reported and handled as AEs. All outcomes of pregnancy must be reported to the Sponsor and the Principal Investigator on the pregnancy outcomes report form.

11.4.2.13 Treatment of overdose

An overdose is a dose in excess of the dose specified for each cohort in this clinical study protocol (CSP).

Over-dosing is not likely to occur in this study since all IP will be administered by site personnel under medical surveillance. In cases of accidental overdose, standard supportive measures should be adopted as required.

An overdose should be documented as follows:

- An overdose with associated AE is recorded as the AE diagnosis/symptoms in the AE Log of the eCRF.
- An overdose without associated symptoms is only reported in the subject's medical records.

11.4.3 *Vital signs*

Systolic and diastolic blood pressure and pulse will be measured in supine position after 10 minutes of rest.

Vital signs will be judged as normal, abnormal, not clinically significant or abnormal, clinically significant. The assessment will be recorded in the eCRF.

Post-IP vital signs, where the change from the values measured at the screening visit is judged as "abnormal, clinically significant" by the Investigator will be reported as AEs.

11.5 Appropriateness of measurements

All methods used for demography and safety assessments are commonly used in standard medical care and in clinical studies.

12 PROCEDURES FOR BIOLOGICAL SAMPLES

12.1 Sample collection

The sample collection procedure for radiation analysis during PET analysis is described in Section 11.2.12.

Clinical laboratory samples are collected according to standard procedures.

12.2 Volume of blood

The estimated volume of blood collected during the study from each subject is presented in Table 12.2-1.

Table 12.2-1 Estimated blood volumes

PART A	**Estimated number of sampling occasions**	**Estimated volume per occasion (mL)**	**Total (mL)**
Clinical chemistry, haematology, microbiology	1	20	20
Blood sampling PET	2	40	80
Total:			**100**
PART B	**Estimated number of sampling occasions**	**Estimated volume per occasion (mL)**	**Total (mL)**
Clinical chemistry, haematology, microbiology	1	20	20
Blood sampling PET	1	40	40
Total:			**60**

12.3 Handling, storage and destruction of laboratory samples

All biological samples will be registered in a biobank at CTC (893), if applicable.

Any remains from radioactive samples drawn and analysed at the PET Centre or the safety laboratory samples will be disposed of immediately after analyses according to standard routines.

12.4 Chain of custody of biological samples

A full chain of custody is maintained for all samples throughout their lifecycle.

CTC keeps full traceability of collected biological samples from the subjects while in storage at the research clinic until shipment and keeps documentation of receipt of arrival.

The sample receiver (the analytical laboratory) keeps full traceability of the samples while in their storage and during use until used or disposed of.

The Sponsor keeps oversight of the entire life cycle through internal procedures, monitoring of study sites and auditing of external laboratory providers.

12.5 Withdrawal of informed consent for donated biological samples

If a subject withdraws consent to the use of biological samples donated, the samples will be disposed of /destroyed, if not already analysed and documented.

The Principal Investigator will ensure that:

- Subject withdrawal of informed consent is notified immediately to Sponsor.
- Biological samples from the subject, if stored at the research clinic, are immediately identified, disposed of/destroyed and the action is documented.

The Sponsor has to ensure that the laboratory(ies) holding the samples is/are informed about the withdrawn consent immediately and that samples are disposed of/destroyed or returned to the research clinic and the action is documented.

13 QUALITY MANAGEMENT, QUALITY ASSURANCE AND QUALITY CONTROL

13.1 Quality management: critical process, system and data identification

During CSP development, the Sponsor will identify those processes, systems (facilities, computerised systems) and data that are critical to ensure human subject protection and the reliability of trial results according to applicable SOPs and International Council for Harmonisation (ICH) E6 R2.

Identified risks will be categorised separately from the CSP.

13.2 Quality assurance and quality control

The Sponsor is responsible for implementing and maintaining quality assurance (QA) and quality control (QC) systems with written SOPs with regards to management of identified

risks, CSP compliance, good clinical practice (GCP) compliance and applicable regulatory requirements.

The Sponsor is responsible for securing agreements with involved subcontractors and to perform regular subcontractor oversight to ensure CSP compliance, GCP compliance and compliance with applicable regulatory requirements.

The Sponsor is responsible for implementing a risk-based validated electronic data capture system and maintain SOPs for the whole life cycle of the system.

QC should be applied to each stage of data handling to ensure that all data are reliable and have been processed correctly.

The Sponsor has delegated the responsibilities outlined above to CTC whilst maintaining overall study oversight.

14 ETHICAL AND REGULATORY REQUIREMENTS

14.1 Ethical conduct of the study

The study will be performed in accordance with ethical principles that have their origin in the Declaration of Helsinki [4] and are consistent with ICH/GCP E6 (R2), EU Clinical Trials Directive, and applicable local regulatory requirements.

14.2 Ethics review

The Principal Investigator is responsible for submission of the CSP, the subject information and ICF, any other written information to be provided to the subjects and any advertisements used for recruitment of subjects to applicable IEC for approval.

Approval must be obtained in writing from the IEC before the first subject can be recruited.

14.3 Subject information and consent

It is the responsibility of the Investigator or an authorised associate to give each potential study subject adequate verbal and written information before any study specific assessments are performed.

The information will include the objectives and the procedures of the study as well as any risks or inconvenience involved. It will be emphasised that participation in the study is voluntary and that the subject may withdraw from participation at any time and for any reason, without any prejudice. All subjects will be given the opportunity to ask questions about the study and will be given sufficient time to consider participation before signing the ICF.

Before performing any study-related procedures the ICF must be signed and personally dated by the subject and by the Investigator. A copy of the subject information including the signed ICF will be provided to the subject.

Documentation of the discussion and the date of informed consent must be recorded in the source documentation and in the eCRF. The subject information sheet and the signed ICF should be filed by the Investigator for possible future audits and/or inspections.

The final approved version of the subject information and ICF must not be changed without approval from the Sponsor and the applicable IEC.

14.4 Subject data protection

The ICF includes information that data will be recorded, collected and processed and may be transferred to European Economic Area (EEA) or non-EEA countries. In accordance with the European Union Data Protection Directive (95/46/EC) and General Data Protection Regulation (GDPR), the data will not identify any persons taking part in the study.

The potential study subject should be informed that by signing the ICF he/she approves that authorised representatives from Sponsor and CTC, the concerned IEC have direct access to his/her medical records for verification of clinical study procedures. For further details on the subject information and ICF process, refer to Section 14.3.

The subject has the right to request access to his/her personal data and the right to request rectification of any data that is not correct and/or complete in accordance with the European Union Data Protection Directive (95/46/EC) and the request will be raised to the Principal Investigator.

The Investigator must file a Subject Identification List which includes sufficient information to link records, i.e. the eCRF and clinical records. This list should be preserved for possible future inspections/audits but must not be made available to the Sponsor except for monitoring or auditing purposes.

Personal data that are collected in the study such as health information and ethnicity are considered as sensitive personal data. This data will be pseudoanonymised, i.e. personally identifiable information (PII) will be removed and replaced by a unique subject ID and will be processed by the Sponsor and other involved parties during the study. After the study end, only anonymised data, i.e. aggregated data sets, can be used.

For this study, the Sponsor Nerudia Ltd is the data controller of all data processed during the study (e.g. Trial Master File [TMF], study reports) and CTC AB is the data processor. Any subcontractors used in the study, are also data processors.

For data that are processed at the clinic(s) (e.g. medical records and ISF), CTC AB is the data controller.

14.5 Changes to the approved clinical study protocol

Any proposed change to the approved final CSP (including appendices) will be documented in a written and numbered clinical protocol amendment. All substantial amendments to the protocol must be approved by the appropriate IEC before implementation according to applicable regulations.

14.6 Audits and inspections

Authorised representatives of Sponsor, or an IEC may perform audits or inspections at the research clinic, including source data verification (SDV). 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, analysed, and accurately reported according to the protocol, ICH-GCP guidelines and any

applicable regulatory requirements. The Investigator will contact the Sponsor immediately if contacted about an inspection at the centre.

14.7 Insurance

Subjects will be covered under Nerudia Ltd's liability insurance policy. The certificate of insurance and an information leaflet containing essential information about the insurance coverage can be provided upon request. The participating subjects are also protected in accordance with national regulations, as applicable. CTC has a company insurance covering services performed by CTC.

15 STUDY MANAGEMENT

15.1 Training of study site personnel

Before enrolment of the first study subject a Sponsor representative or delegate will perform a study initiation visit at the research clinic. The requirements of the CSP and related documents will be reviewed and discussed and the investigational staff will be trained in any study specific procedures and system(s) utilised.

It is the responsibility of the Investigator to ensure that all personnel involved in the study are fully informed of all relevant aspects of the study and have a detailed knowledge of and training in the procedures that are to be executed by them. Any new information of relevance to the performance of this study must be forwarded to the staff involved in a timely manner.

The Investigator will keep a list of all personnel involved in the study together with their function and study related duties delegated. A Curriculum Vitae will be available for all staff to whom study-specific duties are delegated.

15.2 Clinical monitoring

The Sponsor is responsible for securing agreement from all involved parties to ensure direct access to all study related sites, source data/documents, and reports for the purpose of monitoring and auditing by the Sponsor, and inspection by domestic and foreign regulatory authorities.

As defined in the risk-based monitoring (RBM) plan, approved by the Sponsor and provided separately, the responsible Monitor will periodically visit the study site at times agreed upon by the Investigator and the Monitor. At the time of each monitoring visit, the role of the Monitor is (but not limited to) to:

- provide information and support to the investigational team.
- confirm that facilities and resources remain acceptable.
- confirm that the investigational team is adhering to the CSP, applicable SOPs, guidelines, manuals and regulatory requirements.
- verify that data are being accurately and timely recorded in the eCRFs and that IP accountability checks are being performed.
- verify that data in the eCRF are consistent with the clinical records (SDV) in accordance with the RBM plan.

- verify that the correct informed consent procedure has been adhered to for participating subjects.
- ensure that withdrawal of informed consent to the use of the subject's biological samples will be reported and biological samples are identified and disposed of/destructed accordingly, and that this action is documented and reported to the subject.
- verify that AEs are recorded and reported in a timely manner and according to the CSP.
- raise and escalate any serious quality issues, serious GCP breach and any data privacy breach to the Sponsor.

Centralised monitoring will also be performed continuously by study team members at CTC in accordance with the RBM plan.

When the study has been completed and all queries have been resolved and the database has been locked, the Monitor will perform a close-out visit.

15.3 Source data documents

A separate Origin of Source Data List will be generated for each site before start of enrolment, specifying the location of the source of derived information appearing in the eCRF. This document must be signed by the Principal Investigator and the Monitor to confirm agreement before start of recruitment.

Source documents are all documents used by the Investigator or hospital that relate to the subject's medical history, that verifies the existence of the subject, the inclusion and exclusion criteria, and all records covering the subject's participation in the trial. They include laboratory notes, memoranda, material dispensing records, subject files, etc. The eCRF may constitute source data if clearly defined in the Origin of Source Data List.

The Investigator should guarantee access to source documents to the Monitor and the IECs, if required.

15.4 Study agreements

The Principal Investigator must comply with all the terms, conditions, and obligations of the Clinical Study Agreement for this study.

Agreements between Sponsor and CTC must be in place before any study-related procedures can take place, or subjects be enrolled.

15.5 Study timetable and end of study

The study is expected to start in Q1 2020 and to be completed by Q2 2020.

A subject is considered to have completed the study if he/she has completed all visits in the study including the last visit.

The end of the study is defined as the date of the last visit of the last subject in the study.

15.6 Termination of the study

The Sponsor reserves the right to discontinue the study at any time but intends only to exercise this right for valid scientific or administrative reasons.

After such a decision, the PI must inform all participating subjects and perform relevant assessments, preferably according to the scheme for the final assessments. All delivered and unused IP and other study materials must be returned and all eCRFs completed as far as possible.

15.7 Reporting and publication

15.7.1 *Clinical study report*

A clinical study report (CSR), describing the conduct of the study, any statistical analyses performed and the results obtained, will be prepared by CTC and the PET Centre. The report will be reviewed and approved by, as a minimum, the Principal Investigator and the Sponsor.

15.7.2 *Confidentiality and ownership of study data*

Any confidential information relating to the IP or the study, including any data and results from the study, will be the exclusive property of the Sponsor. The Investigator and any other persons involved in the study are responsible for protecting the confidentiality of this proprietary information belonging to the Sponsor.

15.7.3 *Publication*

The results from this study may be submitted for publication at the discretion of the Sponsor.

15.8 Archiving

The Principal Investigator is responsible for maintaining essential documents, (as defined in ICH E6 GCP, Section 8) for 10 years after finalisation of the CSR. This includes any original source documents related to the study, the Subject Identification List (providing the sole link between named subject source records and anonymous eCRF data), the original signed ICFs and detailed records of disposition of IP.

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

The Sponsor will archive the TMF in accordance with ICH E6 GCP, Section 8 and applicable regulatory requirements.

The data from the eCRFs will be sent to the Sponsor and a copy will be sent to the clinic and filed in the Investigator Study File for archiving for 10 years after finalisation of the CSR.

The completed eCRFs are the sole property of the Sponsor and should not be made available in any form to third parties, except for authorised representatives of appropriate Health/Regulatory Authorities, without written permission from the Sponsor.

16 DATA MANAGEMENT

The data management routines include procedures for handling of the eCRF, database set-up and management, data entry and verification, data validation, QC of the database, and documentation of the performed activities including information of discrepancies in the process. The database, data entry screens, and program will be designed in accordance with the CSP.

Data validation/data cleaning procedures are designed to assure validity and accuracy of clinical data. These procedures consist of computerised online edit checks identifying e.g. data values that are outside the allowed range and SAS-programmed batch checks on data exports. All study-specific and standard data validation programming will be tested in a separate testing environment prior to use on production data.

Detailed information on data management will be described in a study-specific Data Management Plan (DMP).

16.1 The web-based eCRF

Clinical data will be entered into a 21 CFR Part 11-compliant eCRF (Viedoc™) provided by PCG Solutions AB. The eCRF includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data will be entered directly from the source documents or at bedside (if the eCRF data constitutes source data). Source data are to be defined at the site before inclusion of the first subject (Section 15.3).

Authorised site personnel designated by the Investigator will complete data collection. Appropriate training and security measures will be completed with the Investigator and all authorised trial site personnel prior to the trial being initiated and any data being entered into the system for any study subject.

16.2 The entering of data into the eCRF

All entries, corrections, and alterations are to be made by the Investigator or designee. Neither the Monitor nor any other study team member besides site staff can enter data in the eCRF. All data should be entered in English. The eCRFs should be completed as soon as possible during or after the subject's visit. The Investigator must verify that all data entries in the eCRFs are accurate and correct. If some assessments are not done, or if certain information is not available, not applicable or unknown, the Investigator or assigned clinical staff should record such information in the eCRF. The Investigator will be required to electronically sign off the clinical data. This will be performed by means of the Investigator's unique User ID and password; date and time stamps will be added automatically at time of electronic signature.

16.3 The query process

The Monitor will review the eCRFs and evaluate them for completeness and consistency. Data in the eCRF will be compared with the respective source documents to ensure that there are no discrepancies for critical data as described in the RBM plan. All entries, corrections,

and alterations are to be made by the Investigator or designee. Neither the Monitor nor any other study team member besides site staff can enter data in the eCRF.

If corrections are needed, queries will be raised within the eCRF, either as a result of built-in edit checks or manually raised by the monitor. An appropriate member of the site staff will answer the queries in the eCRF either by correcting the data or by entering a response to the query. The monitor will either approve the answer/correction or re-issue the query.

16.4 Audit trail

All entries in the eCRF will be fully recorded in a protected audit trail. Once clinical data have been saved, corrections to the data fields will be audit trailed, meaning that the reason for change, the name of the person who made the change, together with time and date will be logged.

16.5 External data

External data consists of data that are not recorded in the eCRF. Data may be received in electronic format or as a paper printout. Key variables are defined in order to uniquely identify each sample record. File and data formats are agreed with the external data provider.

16.6 Medical coding

Medical coding will be performed by trained personnel at CTC. AEs and medical/surgical history verbatim terms will be coded using the Medical Dictionary of Regulatory Activities (MedDRA; latest version available at eCRF finalisation) as specified in the DMP. Prior and concomitant medications will be coded according to the World Health Organisation (WHO) Anatomic Therapeutic Chemical (ATC) classification system. All coding will be approved by Sponsor prior to database lock.

16.7 Database lock

When all data have been entered and discrepancies solved, clean file will be declared, the database will be locked, and the data will be analysed.

17 STATISTICAL METHODS AND DETERMINATION OF SAMPLE SIZE

The principal features of the statistical analysis to be performed are described in this section. A more technical and detailed elaboration of the principal features will be presented in a separate Statistical Analysis Plan (SAP), which will be signed and approved prior to database lock.

Analyses of the primary, secondary and exploratory endpoints will be performed by CTC and the PET Centre.

17.1 General

Continuous data will be presented in terms of evaluable and missing observations, arithmetic mean, standard deviation (SD), median, minimum and maximum value.

Categorical data will be presented as counts and percentages. When applicable, summary data will be presented by treatment, and by assessment time. Individual subject data will be listed by subject number, treatment, and, where applicable, by assessment time.

All descriptive summaries and statistical analyses will be performed using SAS Version 9.4 or later (SAS Institute, Inc., Cary, NC).

Baseline will be defined as the visit with last data collection point prior to the first administration of IP.

No imputation of missing data will be performed.

17.2 Determination of sample size

No formal sample size calculation has been performed for this study. The proposed sample size is considered sufficient to provide adequate information for the study objectives.

Approximately 25 subjects will be screened to achieve 14-17 included and evaluable subjects.

17.3 Analysis data sets

17.3.1 *Full analysis set*

The Full Analysis Set (FAS) will consist of all subjects who have been randomised and received at least one dose of IP and who has at least one post-baseline assessment data.

17.3.2 *Safety analysis set*

The Safety Analysis Set will comprise all subjects who received at least one dose of the IP.

17.3.3 *Per protocol set*

The Per Protocol Set (PPS) will consist of all subjects who have been randomised and completed the study without any major protocol deviations that are judged to compromise the analysis of the data. All protocol violations will be judged as major or minor prior to database lock.

17.4 Description of study population

17.4.1 *Demographics and baseline characteristics*

Descriptive statistics for demographics, weight and height will be presented by treatment and study part.

17.4.2 *Medical/surgical history and prior/concomitant medication*

Medical/surgical history will be presented by system-organ-class (SOC) and preferred term (PT). Prior/concomitant medications will be presented by ATC level 1, 3 and 5.

All data will be listed by subject.

17.4.3 *Treatment compliance*

The number of subjects treated with each formulation in each part will be listed.

17.4.4 *Physical examination*

Clinically significant and non-clinically significant abnormal findings will be specified and presented by subject and summarised by treatment and study part.

All data will be listed by subject.

17.4.5 *12-lead ECG*

All ECGs will be categorised as ”normal”, ”abnormal, not clinically significant”, or ”abnormal, clinically significant” (as judged by the Investigator) and summarised by treatment and study part using frequency tables.

All data will be listed by subject.

17.4.6 *Clinical laboratory analyses*

Clinical laboratory data will be summarised by treatment and study part.

Abnormal, clinically significant values will be summarised separately if considered appropriate.

All data will be listed by subject.

17.5 Analysis of PET endpoints

The PET Centre will be responsible for evaluation of PET data, and present time activity data on deposition during the dynamic scans in PET-scan 1 and 2. For the evaluation of [^{11}C]nicotine kinetics in the lung, primary bronchi, oral cavity and airways, volumes of interest (VOIs) will be delineated, and time activity data generated utilising PET-image analysis software. The VOIs will be delineated based on the anatomical information from the low dose CT-images combined with the uptake seen in the PET images. Deposition in the lungs will be measured and presented for one large VOI covering the entire lung.

Furthermore, a small VOI may be delineated to be representative for deep lung tissue, however this VOI will not include the entire deep lung tissue. Even though alveolar tissue cannot be identified in detail on the ULDCT or PET images, the highest density of alveolar tissue, combined with lowest density of bronchi and major blood vessels, will be found in the peripheral part of the lung and a small VOI will be delineated at a position representative for deep lung tissue.

For PET-scan 3 acquired over the brain, the amount of nicotine distributed to the brain will be estimated. A whole brain VOI will be delineated on the T1-MR images overlaid on the brain PET-image. This part of the study is explorative and dependent on the time course of the [^{11}C]nicotine uptake to the blood and the time activity uptake for brain nicotine may be difficult to measure with a sufficient signal-to-noise ratio.

The uptake data will be stored in spreadsheet format and sent to study sponsor to enable further data analysis. Furthermore, and as an exploratory part of the PET-image analysis, other regions may be delineated and analysed, if visual inspection of overlaid PET and ULDCT images shows substantial uptake in other tissues or regions.

Input data for evaluation of [^{11}C] uptake and distribution:

- Amount of radioactivity administered from inhalator (inhalator measured before and after inhalation)
- PET-images showing deposition of radioactivity in the lung
- PET-images showing deposition of radioactivity in the oral cavity
- Radioactivity measured in arterial blood.

The relative amount of radioactivity that is deposited in the lung can be determined from the activity measured in the PET images and the known amount of activity in the inhalator before and after the inhalation.

Blood radioactivity data will be collected processed and stored in excel spreadsheets.

Time course of delivered nicotine as well as fraction that reach lung or is deposited in oral cavity, throat and large bronchi will be tabulated and sent to the study sponsor as excel files. The radioactivity measured continuously in arterial blood as well in the discrete blood samples will tabulated in excel sheets and sent to the study sponsor.

17.6 Analysis of safety endpoints

17.6.1 *Adverse events*

An overview of all AEs, including SAEs, intensity, relationship to IP, and deaths will be presented by SOC and PT.

Incidence of AEs and SAEs will be summarised by SOC and PT by treatment and study part and overall.

All AE data will be listed subject and include the verbatim term entered by the Investigator.

17.6.2 *Vital signs*

Vital signs (systolic/diastolic blood pressure and pulse) will be summarised by treatment and study part. Data will be presented with absolute and percent change from baseline.

All data will be listed by subject.

18 REFERENCES

1. Bergström M, Nordberg A, Lunell E, Antoni G, Långström B. Regional deposition of inhaled 11C-nicotine vapor in the human airway as visualized by positron emission tomography. Clin Pharmacol Ther 57:309-317, 1995
2. Berridge MS, Apana SM, Nagano KK, Berridge CE, Leisure GP, Boswell MV. Smoking produces rapid rise of [11C]nicotine in human brain. Phychopharmacology (Berl) 298:383-394, 2010
3. National Cancer Institute Cancer Therapy Evaluation Program. Common terminology criteria for adverse events, CTCAE v5.0 (2017).
4. Declaration of Helsinki: https://www.wma.net/policies-post/wma-declaration-of-helsinki-ethical-principles-for-medical-research-involving-human-subjects/

19 SIGNATURES

19.1 Principal Investigator statement

I have read and understood this CSP and agree to conduct the study accordingly and to comply with the Investigator obligations stated in this CSP, GCP and applicable regulatory requirements.

Principal Investigator

[redacted]

CTC Clinical Trial Consultants AB

Site

19.2 Signature page (approval of the clinical study protocol)

Sponsor signatories