Protocol ACH-CYT-01

A Phase 1 Open Label, Randomized, Two-Way Crossover Study in Healthy Volunteers to Investigate the Effect of Food on the Bioavailability of Cytisine

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Version 1.0

CONFIDENTIAL

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SPONSOR SIGNATURE PAGE

PROTOCOL NUMBER	**ACH-CYT-01**
PROTOCOL TITLE	A Phase 1 Open Label, Randomized, Two-Way Crossover Study in Healthy Volunteers to Investigate the Effect of Food on the Bioavailability of Cytisine
EudraCT NUMBER:	2017-001562-19
PLANNED STUDY DOSE:	2 x 1.5 mg cytisine tablets
PROTOCOL VERSION	**1.0**
PROTOCOL DATE	19 May 2017

Approvals:

[redacted]

19 May 2017
Date

[redacted]

19 May 2017
Date

[redacted]

19 May 2017
Date

[redacted]

19 May 2017
Date

SYNOPSIS

NAME OF COMPANY: Achieve Life Sciences, Inc

NAME OF INVESTIGATIONAL MEDICINAL PRODUCT (IMP):
Cytisine 1.5 mg film-coated tablet

TITLE OF STUDY: A phase 1 open label, randomized, two-way crossover study in healthy volunteers to investigate the effect of food on the bioavailability of cytisine

CHIEF INVESTIGATOR: [redacted]

STUDY CENTRE: Simbec Research Ltd (Simbec)
Merthyr Tydfil, CF48 4DR, UK

CLINICAL PHASE: I

OBJECTIVES:

Primary:

- To compare the bioavailability (C_{max} and $AUC_{0-\infty}$) of cytisine in healthy subjects under fed and fasted conditions, following administration of 3 mg cytisine (2 x 1.5 mg cytisine tablets).

Secondary:

- To compare the AUC_{0-t}, $T_{½}$ and T_{max} of cytisine in subjects under fed and fasted conditions, following administration of 3 mg cytisine (2 x 1.5 mg cytisine tablets).
- To assess the safety and tolerability of cytisine at the 3 mg dose level under fed and fasted conditions.
- To assess the renal elimination of cytisine via measurement of urinary concentrations of cytisine.
- To explore possible effects of other study parameters on the bioavailability of cytisine (e.g. gender, BMI).
- To explore for cytisine effects on QT/QTc interval prolongation and cardiac safety.

METHODOLOGY:

This will be an open-label, randomised, 2-period, single-dose crossover study to determine the comparative bioavailability of cytisine following single-dose administration in healthy male and female subjects under fed and fasted conditions.

The study will be comprised of a pre-study screen, followed by 2 treatment periods (1 and 2) and a post-study follow-up.

Screening (Day -28 to Day -2): Screening assessments will be carried out within 28 days before first administration of IMP. Eligible subjects will be asked to return for the treatment periods. Continued eligibility will be confirmed pre-dose during each treatment period.

Treatment Periods (Day -1 to Day 2): Eligible subjects will receive a single-dose of IMP under fed and fasted conditions over 2 treatment periods.

Each treatment period will be approximately 2 days' duration and will require 2 overnight stays in the Clinical Unit. Each period will begin the afternoon before dosing on Day -1 until 24 hours

(h) postdose (Day 2).

During each treatment period, subjects will arrive at the Clinical Unit on Day -1 and will undergo an overnight fast of at least 10 h prior to dosing on Day 1. Holter monitoring will also be initiated. Randomisation will occur on the morning of Day 1, Period 1, at least 1 hour prior to dosing, and IMP will be administered under fasted (after an overnight fast of at least 10 h) or fed (after a high fat breakfast) conditions. Subjects will be discharged 24 h postdose (Day 2). Pharmacokinetic (PK) samples (plasma and urine) will be collected predose and up to 24 h postdose (Day 2) during each period (17 plasma and 6 urine samples per period) for the measurement of plasma and urine cytisine concentrations. Safety will also be evaluated at specified times.

Continuous ECG measurements will be obtained via Holter monitor that will be initiated on Day-1 prior to dosing and continue until approximately 24 hours post dosing.

Final blood draw for repeat haematology and chemistries will be obtained just prior to discharge on Day 2.

There will be at least 72 hours between dose administrations.

Post Study: Post study telephone follow-up will be conducted 6 to 8 days after last dose of IMP.

NUMBER OF SUBJECTS: 24 subjects (approximately 12 males and 12 females) will be randomised/enrolled to complete the study.

MAIN INCLUSION CRITERIA:

Healthy males or females (non-pregnant/non-lactating) aged 18 - 55 years, with no allergy or sensitivity to cytisine or any of its excipients, who unless surgically sterile, abstaining from sexual intercourse or of non-child bearing potential, are willing to use an effective method of contraception from the first dose and for 3 months after the last dose of IMP.

IMP ADMINISTRATION:

Each subject will receive the IMP over 2 treatment periods under fed and fasted conditions in accordance with the randomisation schedule:

Schedule A (12 subjects):

- Period 1: Cytisine (2 x 1.5 mg tablets) will be administered 30 minutes after the start of a high fat breakfast (fed state).
- Period 2: Cytisine (2 x 1.5 mg tablets) will be administered after an overnight fast of at least 10 hours (fasting state).

Schedule B (12 subjects):

- Period 1: Cytisine (2 x 1.5 mg tablets) will be administered after an overnight fast of at least 10 hours (fasting state).
- Period 2: Cytisine (2 x 1.5 mg tablets) will be administered 30 minutes after the start of a high fat breakfast (fed state).

240 mL water will be given with the dose.

CRITERIA FOR EVALUATION:

PK:

PK Endpoints:

Plasma: The following PK endpoints will be derived from plasma cytisine concentration versus time data following administration of 3 mg cytisine (2 x 1.5 mg) under fed and fasted conditions: Maximum concentration (C_{max}), time to C_{max} (T_{max}), elimination rate constant (k_{el}), terminal

elimination halflife ($t_{1/2}$), area under the concentration versus time curve (AUC) from time of dosing to last measurable concentration (AUC_{0t}), AUC extrapolated to infinity (AUC_{0inf}) and $AUC_{\%\ extrapolated}$ (residual area).

Urine: Amount excreted in urine over time (Ae) and percent of drug excreted in urine (Ae%) will be calculated.

Safety:

Safety Endpoints: AEs, vital signs (supine systolic/diastolic pressure, pulse and oral temperature), 12 lead ECG (heart rate, PR interval, QRS width, QT interval and QT interval corrected using Fridericia's (QTcF interval) formula), laboratory safety (haematology, biochemistry and urinalysis).

STATISTICAL METHODS:

All statistical analysis will be performed using SAS® version 9.1.3 or higher.

Demographic and Background Data: All demographic and background data will be listed. Demographic data will be summarised descriptively (age, height, weight and BMI) by gender and overall subject disposition and analysis sets (safety, PK) will also be listed and summarised by frequency.

PK: All plasma and urine cytisine concentrations and derived PK data following administration of cytisine (2 x 1.5 mg) under fed and fasted conditions will be listed and the PK data summarised descriptively. Individual and mean concentration time data will also be plotted.

Following logarithmic transformation C_{max}, AUC_{0-t} and AUC_{0-inf} values will be subjected to an analysis of variance (ANOVA) including fixed effects for sequence, period, treatment and subject nested within sequence. Point estimates and 90% confidence intervals (CI) will be constructed for the contrasts between treatments using the residual mean square error obtained from the ANOVA. The point and interval estimates will be back-transformed to give estimates of the ratios of the geometric least squares means (LSmeans) and corresponding 90% CI. Estimated geometric means will also be presented for each treatment. An assessment of T_{max} will be performed using the Wilcoxon Signed-Rank test. In addition, the Hodges-Lehman estimate of the median difference in T_{max} and corresponding 95% CI will be presented.

Safety: All safety data will be listed.

AEs will be coded using Medical Dictionary for Regulatory Activities (MedDRA) version 20.0. The incidence of treatment emergent AEs (TEAE) will be summarised by organ system, preferred term, severity and relationship to study drug. Abnormal laboratory safety results will be listed.

QT/QTc: Triplicate ECGs, extracted at pre-dose and 0.5h, 1h, 2h, 3h, 4h, 6h, 8h, 12h and 24h post dose for both Period 1 and Period 2 obtained from the Holter recordings will be listed and analysed. Recordings collected from Day -1 to pre-dose may be analysed if large heart rate effect is observed during the trial.

DURATION OF STUDY:	Approximately 6 weeks for each individual (from screening to post-study follow-up).

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LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

ABBREVIATION/TERM	DEFINITION
ADR	Adverse Drug Reaction
AE	Adverse Event
ANOVA	Analysis of Variance
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
AUC_{0-t}	AUC from Time Zero to Last Sampling Time with Quantifiable Concentrations
$AUC_{0-\infty}$	AUC from Time Zero to Infinity
%AUC	Residual Area or Percentage of Extrapolated Part of $AUC_{0-\infty}$
BMI	Body Mass Index
C_{last}	Last Quantifiable Concentration
C_{max}	Maximum Observed Plasma Concentration
CRF	Case Report Form
CV%	Coefficient of Variation
ECG	Electrocardiogram
EMA	European Medicines Agency
GCP	Good Clinical Practice
GMP	Good Manufacturing Practice
GMR	Geometric Means Ratio`
ICH	International Conference on Harmonization
IMP	Investigational Medicinal Product (for this protocol indicates cytisine 1.5 mg film coated tablet)
LC/MS/MS	Liquid Chromatography-tandem Mass Spectrometry
LLOQ	Lower Limit of Quantification
MedDRA	Medical Dictionary for Regulatory Activities
PK	Pharmacokinetics
RBC	Red Blood Cell Count
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SD	Standard Deviation
SOC	MedDRA System Organ Class
T_{max}	Time to Maximum Observed Concentration
$t_{1/2}$	Apparent Terminal Elimination Half-life
UADR	Unexpected Adverse Drug Reaction
UAE	Unexpected Adverse Event
ULOQ	Upper Limit of Quantification
WBC	White Blood Cell Count
λ_z	Apparent Terminal Elimination Rate Constant

1. ETHICS

1.1. Research Ethics Committee

This study protocol will be submitted to the Research Ethics Committee (REC) for review and approval. The approval of the REC must be obtained before commencement of any study procedures.

The favourable opinion is conditional upon the Sponsor registering the clinical trial in a publicly accessible database, within 6 weeks of the first participant recruited.

All substantial protocol amendments must be approved by the REC responsible for the study. Non-substantial amendments will not require prior approval by the REC.

If the study is stopped due to adverse events (AEs), it will not be recommenced without reference to the REC responsible for the study.

The outcome of the study (e.g. completed) will be reported to the REC responsible for the study within 90 days of completion of the last subject's final study procedures. In the event of the study being prematurely terminated, a summary safety report will be submitted to the REC responsible for the study within 15 days.

A summary of the clinical study report will be submitted to the REC responsible for the study within 1 year of completion of the last subject's final study procedures.

The REC will be informed that [redacted] is a commercial organisation and that the study is funded by Achieve Life Science. The subjects who take part in the clinical study will be paid for their inconvenience and have been informed that there will be no benefits gained by their participation. All potential conflicts of interest will be declared by the Investigators.

1.2. Subject Information and Consent

Potential subjects who volunteer for participation in the study will be informed of the aims, methods, anticipated benefits and potential hazards of the study and any possible discomfort it may entail. Information will be given in both oral and written form and in the manner deemed appropriate by the Clinical Unit standard operating procedures (SOPs). Each subject will also be informed of his/her right to withdraw from the study at any time, for any reason.

A written explanation (participant information sheet) and informed consent form will be provided and the subject will be allowed sufficient time to consider the study information. Prior to signing the informed consent form, the subject will be given an opportunity to discuss any issues concerning the study with an Investigator who has suitable knowledge of the study and will have all questions answered openly and honestly.

If the subject is willing to participate in the study, the informed consent form will be signed and personally dated by the subject and the person taking consent. The subject will receive a copy of the informed consent form together with the participant information sheet and the original signed informed consent form will be retained with the study records at the Investigator site. In addition, the actions and completion of the consenting process will be recorded in the subject's medical record (i.e., source document).

1.3. Indemnity Arrangements

The Sponsor and [redacted] carry insurance to pay compensation for injury, accident, ill health or death caused by participation in this study without regard to proof of negligence in accordance with the insurance and compensation in the event of injury in phase I clinical trials 2012, guidance issued by the ABPI, the BioIndustry Association and the Clinical Contract Research Association in consultation with the Department of Health and the National Research Ethics Service.

2. INTRODUCTION AND BACKGROUND

2.1. Smoking and Health

Tobacco smoking contributes to some 5 million premature deaths each year worldwide.[1] Smoking is highly addictive, with more than 95% of unaided attempts at cessation failing to last 6 months.[2] Every year that a smoker delays quitting beyond the mid-30s, it has been estimated that the person loses 3 months of life expectancy.[3] The World Health Organization's Framework Convention on Tobacco Control identifies evidence-based approaches to promote smoking cessation, which include mass-media campaigns, tax increases on tobacco, and help for smokers wanting to stop.[4]

2.2. Cytisine

[redacted] containing the active substance cytisine has been licensed and marketed in Central and Eastern Europe for several decades by [redacted]), but remains relatively unknown outside Eastern Europe despite requests for market licensing worldwide.[5-7]

Cytisine's mechanism of action has assisted basic pharmacologists in understanding the complex pharmacology of the various subtypes of the nicotinic acetylcholine receptor.[8] These studies have shown that both nicotine and cytisine bind strongly and preferentially to pre-synaptic $\alpha_4\beta_2$ receptors[9,10] that mediate the release of dopamine in the shell of the *nucleus accumbens*. This receptor subtype has been implicated in the development and maintenance of nicotine dependence[11] and was the primary target for the drug varenicline, which has proved effective in aiding smoking cessation.[12]

Two studies have evaluated the pharmacokinetics of cytisine in man. Study CYT–BIO-02-07 was conducted according to the principles of Good Clinical Practice (GCP) and sponsored by [redacted] The pharmacokinetic profile of cytisine in plasma was determined following administration of 1.5 mg cytisine (in the form of a [redacted] tablet) in 36 male volunteers. All drug administrations were given in the fasted state. The main outcomes are summarised in the table below and are as follows:

Table 1: CYT-BIO-02-07 PK Results

C_{max}	15.55 ng/mL
AUC_{0-t}	59.80 ng.h/mL
$AUC_{0-\infty}$	73.63 ng.h/mL
T_{max}	0.92 h (≈ 55 min)
$T_{½}$	4.09 h

- The mean C_{max} **(**n=36) was 15.55 ± 6.55 ng/ml (95% CI: 13.33 - 17.76).
- The concentration-time curves over 0-t period (AUC_{0-t}) for cytisine was 59.80 ng*hr/ml ± 16.77 (95% CI: 54.13 - 65.48).
- The $AUC_{0-\infty}$ was 73.63 ng*hr/ml ± 21.27 (95% CI: 66.43 - 80.83).
- Time to maximal plasma concentration (Tmax) ranged from 0.33 h to 1.75 h with the mean T_{max} value at 0.92 h ± 0.40 (95% CI: 0.78 ÷ 1.06).
- The plasma elimination half-life ranged from 2.74 h to 6.36 h with a mean of 4.09 h ± 0.82 (95% CI: 3.81 - 4.37).
- Of note: cumulated cytisine levels excreted in the urine during the 24 hr indicated that ~64% of administered dose was excreted as parent compound (34.71% - 100.24%).

A second study[13] reported the pharmacokinetic profile of cytisine in seven healthy subjects (aged between 20 and 39 years) following administration of 3 mg cytisine (in the form of a [redacted] tablet). Subjects were eligible if they were current cigarette smokers at the time of the study and on average, the subjects smoked 10.6 cigarettes per day. There was no restriction on diet or smoking during the study. The main outcomes are summarised in the table below and are as follows:

Table 2: Single Dose PK Results

C_{max}	27.76 ng/mL
$T_{½}$	4.8 h
T_{max}	1-2 h
VD	115 L
CL	16.7 L/h

- The peak concentrations (C_{max}) of cytisine were between 23.37 and 32.04 ng/mL. The mean C_{max} was 27.76 ng/mL.

- Plasma elimination half-life was calculated to be 4.8 h.
- Peak plasma concentrations were typically observed at 2 h after administration. In two subjects, the peak plasma concentration was observed at 1 h post-dose, suggesting that the peak plasma concentration may actually have been achieved between 1 and 2 h for all subjects.
- Of note: cytisine was still detectable in the urine collected at 24 h for all subjects (mean 24 h concentration was 428.15 ng/mL).
- Data together in this study were modeled using NONMEM to estimate the volume of distribution (VD) at 115 L and clearance (CL) at 16.7 L/h.

3. RATIONALE FOR THE STUDY

No studies in man have been conducted to examine the pharmacokinetic profile of cytisine in the fed versus fasted state. A recent bioavailability study in a limited number of dogs, where cytisine was given in the fed and fasted states, indicated a potential 28% increase in C_{max} occurred in the presence of food. Therefore, this study will compare the pharmacokinetics of cytisine in the fed and fasted state in 24 healthy volunteers to more clearly determine to what level a potential food effect (Fed state) might occur with cytisine administration and whether dietary restrictions need to be defined in a future Phase 3 trial design for cytisine. In addition, assessment for any potential effect of cytisine on QT/QTc interval prolongation or other cardiac safety measures will be explored and analyzed in relationship to the cytisine pharmacokinetic profile.

4. STUDY OBJECTIVES

4.1. Primary Objective

1. To compare the bioavailability (C_{max} and $AUC_{0-\infty}$) of cytisine in subjects under fed and fasted conditions, following administration of 3 mg cytisine (2 x 1.5 mg cytisine tablets).

4.2. Secondary Objectives

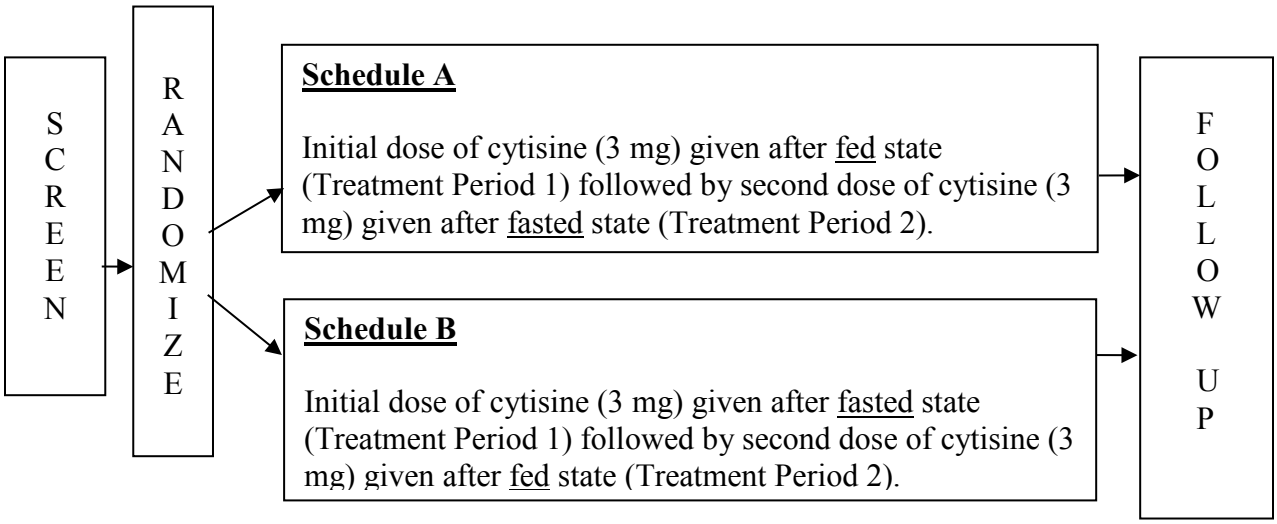
1. To compare the AUC_{0-t}, $T_{½}$ and T_{max} of cytisine in subjects under fed and fasted conditions, following administration of 3 mg cytisine (2 x 1.5 mg cytisine tablets).
2. To assess the safety and tolerability of cytisine at the 3 mg dose level under fed and fasted conditions.
3. To assess the renal elimination of cytisine via measurement of urinary concentrations of cytisine.
4. To explore possible effects of other study parameters on the bioavailability of cytisine (e.g. gender, BMI).
5. To explore for cytisine effects on QT/QTc interval prolongation and cardiac safety.

5. INVESTIGATIONAL PLAN

5.1. Study Design

This will be an open-label, randomised, 2-period, single-dose crossover Phase I study conducted in healthy volunteers as shown below:

Figure 1: Study Design Overview



The study will be comprised of a pre-study screen, followed by 2 treatment periods (1 and 2) and a post-study follow-up.

Screening (Day 28 to Day 2): Screening assessments will be carried out within 28 days before first administration of IMP. Eligible subjects will be asked to return for the treatment periods. Continued eligibility will be confirmed pre-dose during each treatment period.

Treatment Periods (Day -1 to Day 2): Eligible subjects will receive a single-dose of IMP under fed and fasted conditions over 2 treatment periods.

- Each treatment period will be approximately 2 days' duration and will require 2 overnight stays in the Clinical Unit. Each period will begin the afternoon before dosing on Day -1 until 24 hours (h) postdose (Day 2).
- During each treatment period, subjects will arrive at the Clinical Unit on Day -1 and will undergo an overnight fast of at least 10 h prior to dosing on Day 1. Holter monitoring will also be initiated. Randomisation will occur on the morning of Day 1, Period 1, at least 1 hour prior to dosing, and IMP will be administered under fasted (after an overnight fast of at least 10 h) or fed (after a high fat breakfast) conditions and subjects will be discharged 24 h postdose (Day 2).
- Pharmacokinetic (PK) samples (plasma and urine) will be collected predose and up to 24 h postdose (Day 2) during each period (17 plasma and 6 urine samples per period) for the measurement of plasma and urine cytisine concentrations. Safety will also be evaluated at specified times.

- Continuous ECG measurements will be obtained via the Holter monitor initiated on Day -1 and continued until approximately 24 hours post dosing.
- Final ECGs and blood draw for repeat haematology and chemistries will be obtained just prior to discharge on Day 2.
- There will be at least 72 hours between dose administrations.

Post Study: Post study telephone follow-up will be conducted 6 to 8 days after last dose of IMP. If any adverse events are recorded at post study telephone assessment, arrangements will be made with the subject, so that they are followed-up appropriately and the final outcome determined.

The end of study is defined as completion of safety follow-up on all subjects. The maximum duration of this study is estimated to be around 6 weeks (screening to last subject's last visit).

5.2. Discussion of Study Design

This study design minimizes the influence of inter-subject variability, which is assumed to be higher than the intra-subject variability, as each subject will be administered cytisine in both the fed and fasted states. As each subject serves as his own control, the precision of the comparison should be improved.

The blood samples for the determination of cytisine plasma concentrations will be collected prior to and up to 24 hours after cytisine administration. Taking into account the known terminal elimination half-life of cytisine (about 4 hours), this sampling schedule is expected to provide a reliable estimate of the extent of exposure.

The washout of at least 72 hours between dose administration will allow a complete elimination of cytisine between doses and is deemed sufficiently long to avoid any relevant carry-over effect.

6. SELECTION OF STUDY POPULATION

24 subjects (approximately 12 males and 12 females) will be required to complete the study.

The study is to be conducted in healthy subjects and therefore participants are not expected to derive any therapeutic benefit from taking part. A healthy subject population with carefully considered inclusion/exclusion criteria will avoid the potential for interaction of cytisine with any underlying disease state or concomitant medication that might be necessary to administer to patients, while ensuring that subjects are fit and well enough for participation in the study.

The following eligibility criteria are designed to select subjects for whom protocol treatment and procedures are considered appropriate. All relevant medical and non-medical conditions should be taken into consideration when deciding whether this protocol is suitable for a particular subject.

6.1. Inclusion Criteria

6.1.1. To be Confirmed at Screening

1. Healthy males and females between 18 and 55 years of age.

a. If a female subject of child bearing potential, a negative pregnancy test at screening and admission and willing to use an effective method of contraception (unless of non-childbearing potential or where abstaining from sexual intercourse is in line with the preferred and usual lifestyle of the subject) from first dose until 3 months after last dose of IMP.
b. If a female subject of non-child bearing potential, a negative pregnancy test at screening and admission. For the purposes of this study, this is defined as the subject being amenorrheic for at least 12 consecutive months or at least 4 months post-surgical sterilisation (including bilateral fallopian tube ligation or bilateral oophorectomy with or without hysterectomy). Menopausal status will be confirmed by demonstrating at screening that levels of follicle stimulating hormone (FSH) fall within the respective pathology reference range. In the event a subject's menopause status has been clearly established (for example, the subject indicates she has been amenorrheic for 10 years), but FSH levels are not consistent with a post-menopausal condition, determination of subject eligibility will be at Investigator's discretion following consultation with the Sponsor.
c. If a male subject, willing to use an effective method of contraception (unless anatomically sterile or where abstaining from sexual intercourse is in line with the preferred and usual lifestyle of the subject) from first dose until 3 months after last dose of IMP.

2. Subject with a body mass index (BMI) of 18-32 kg/m^2. BMI = body weight in kg / $[\text{height in m}]^2$.
3. Subject with no clinically significant abnormal serum biochemistry, haematology and urine examination values within 28 days before the first dose of IMP.
4. Subject with negative urinary drugs of abuse screen, determined within 28 days before the first dose of IMP (a positive alcohol or cotinine result may be repeated at Investigator's discretion).
5. Subject with negative human immunodeficiency virus (HIV), hepatitis B surface antigen (Hep B) and hepatitis C virus antibody (Hep C) results.
6. Subject with no clinically significant abnormalities in 12-lead ECG determined after minimum of 5 minutes in supine position within 28 days before the first dose of IMP.
7. Subject with no clinically significant abnormalities in vital signs (systolic blood pressure between 90-150 mmHg, diastolic blood pressure (DBP) between 50 and 90 mmHg, and pulse rate (PR) between 40-110 bpm, measured on the dominant arm after minimum of 5 minutes in supine position) determined within 28 days before first dose of IMP.
8. Subject must be available to complete the study (including post study follow-up) and comply with study restrictions.
9. Subject must provide written informed consent to participate in the study.

6.1.2. To be Re-Confirmed Prior to Dosing

1. Subject continues to meet all screening inclusion criteria (before the first dose only).
2. Subject has a negative urinary drugs of abuse screen (including alcohol & cotinine).

3. Female subject has a negative pregnancy test.

6.2. Exclusion Criteria

6.2.1. To be Confirmed at Screening

1. Known hypersensitivity/allergy reaction to varenicline, other cytisine-derivatives or any of the excipients in the [redacted] formulation [redacted]).
2. History of severe hypersensitivity reactions to any other drugs.
3. History of any medical condition (e.g. gastrointestinal, renal or hepatic) or surgical condition (e.g. cholecystectomy, gastrectomy) that may affect drug pharmacokinetics (absorption, distribution, metabolism or excretion).
4. Difficulty in donating blood on either arm or known history.
5. History of alcoholism or drug abuse within last 2 years.
6. Regular nicotine intake (e.g., smoking, nicotine patch, nicotine chewing gum or electronic cigarettes) within previous 3 months and inability to refrain from nicotine intake from Screening until end of study.
7. Use of prescription or non-prescription drugs, including vitamins, herbal and dietary supplements within 14 days (or 5 half-lives, whichever is longer) prior to the first dose of IMP, unless in the opinion of the Investigator the medication will not interfere with the study procedures or compromise subject safety.
8. Participated in any investigational drug clinical trial within the previous 3 months or a marketed drug trial within the previous 30 days prior to randomization on Day 1 of Period 1.
9. Donation of 450 mL or more blood or had history of significant blood loss due to any reason or had plasmapheresis within 3 months before the first dose of IMP.
10. Any special food restrictions that may hinder ability to consume the high fat breakfast provided during the study; such as lactose intolerance, vegan, low-fat, low sodium, etc.
11. Any inability or difficulty in fasting.
12. Inability to communicate well with Investigators (i.e., language problem, poor mental development or impaired cerebral function).
13. Any other condition that the Principal Investigator considers making the subject unsuitable for this study.

6.2.2. To be Re-Confirmed Prior to Dosing:

1. Development of any exclusion criteria since screening (before the first dose only).
2. Use of prescription or non-prescription drugs, including vitamins, herbal and dietary supplements since screening, unless in the opinion of the Investigator the medication will not interfere with the study procedures or compromise subject safety.
3. Participation in a clinical study since the screening visit (before the first dose only).

4. Donation of 450 mL or more blood since the screening visit (before the first dose only).

6.3. Removal of Subjects from Therapy or Assessment

Each subject will be informed of their right to withdraw from the study at any time and for any reason.

An Investigator will withdraw a subject from the study at any time for any of the following reasons:

- If a subject experiences a serious or intolerable AE, that prevents them from continuing.
- If a subject incurs a significant protocol violation which impacts on their safety or the scientific integrity of the study (this will be discussed on a case-by-case basis with the Sponsor).
- At the request of the Sponsor.
- If it is considered that the subject's health is compromised by remaining in the study or the subject is not sufficiently cooperative.
- If a subject is lost to follow-up.

The reasons for any subject withdrawal will be recorded on the study completion form of the case report form (CRF).

If a subject is withdrawn or chooses to withdraw from the study for any reason, every possible effort will be made to perform the evaluations described for the post-study follow-up. The data collected from withdrawn subjects will be included in the study report.

In the event of any abnormalities considered to be clinically significant, subjects will be followed up with appropriate medical management until values are considered to be clinically acceptable. Referral or collaborative care will be organised if considered necessary.

Since 24 subjects are required to complete the study, subjects who withdraw from the study before receiving any study medication will be replaced. Subjects who are withdrawn from the study due to significant drug-related AEs will not be replaced. Replacement of all other subjects withdrawn from the study after receiving study medication will be decided on a -case-by-case basis by the Chief Investigator (or deputy) and Sponsor.

7. RETRICTIONS PRIOR AND DURING THE STUDY

To avoid possible negative effects on the measurement of plasma drug concentrations and/or on subject's safety, several restrictions are to be adopted as outlined in the eligibility criteria and in the following sections.

7.1. Confinement

Each subject will complete 2 treatment periods (Period 1 and Period 2). Each period will require 2 nights stay (arrive at the unit in the afternoon of Day -1 and discharged 24 hours post dose on Day 2).

7.2. Diet and Fluid Restrictions

7.2.1. Meal Times

Fasting:

- Subjects will fast overnight for at least 10 hours before dosing.
- Lunch will be served: approximately 4 h post-dose.
- Dinner will be served: approximately 8 h post-dose.
- Snack will be served: approximately 12 h post-dose.

Fed:

- Subjects will fast overnight for at least 10 hours.
- A high fat breakfast will be served at 30 min pre-dose (breakfast needs to be finished at least 5 minutes before dosing).
- Lunch will be served: approximately 4 h post-dose.
- Dinner will be served: approximately 8 h post-dose.
- Snack will be served: approximately 12 h post-dose.

The example of a high fat (approximately 50% of total caloric content of the meal) and high-calorie (approximately 800-1000 calories) breakfast provided in the US Food and Drug Administration guidance document for food-effect bioavailability and fed bioequivalence studies (Guidance for Industry, U.S. Department of Health and Human Services Food and Drug Administration Center for Drug Evaluation and Research (CDER), December 2002) is approximately 150, 250 and 500-600 calories from protein, carbohydrate and fat respectively. Suggested content to be: 2 eggs fried in butter, 2 strips of bacon, 2 slices of toast, 4 ounces of hash brown potatoes and 8 ounces of whole milk. Consumption of the standardized breakfast will be documented.

7.2.2. Fluid Intake

No fluids (apart from water taken with dose) are allowed from 1 h prior to dosing until 1 h afterwards. Water is then allowed ad libitum. Decaffeinated tea and coffee as well as squash/cordial are allowed from 4 h post-dose.

7.2.3. Alcohol Intake

The consumption of alcohol will be limited to a maximum of 2 units per day from 7 days prior to the first dose of IMP. Alcohol will be avoided completely for at least 2 days prior to the first dose of IMP and throughout the study period. Any deviation outside this alcohol intake restriction will be assessed on a case-by-case basis at Investigator's discretion (provided the subject's alcohol intake will not impact in the safety aspects and objectives of the study and the subject has a negative alcohol screen prior to dosing).

7.2.4. Caffeine

Food or drink containing caffeine, including coffee, tea, cola, energy drinks or chocolates will be avoided completely for 2 days prior to dosing and whilst the subjects are resident in the Clinical Unit (during each dosing period).

7.2.5. Poppy Seeds

Subjects must not eat food containing poppy seeds for 3 days before each visit to the Clinical Unit, as consumption of poppy seeds can lead to a positive opiate result in the drugs of abuse test.

7.2.6. Grapefruit Juice and Other Restrictions

No food or drink containing grapefruit, cranberry, or Seville oranges (including marmalade and fruit juices), and/or food or drink, sweets, candies or other confectionary containing liquorice will be allowed from 7 days before the first dose of IMP until the final study visit.

Each subject will be questioned on the specific points at admission to the clinical unit (Day -1 of each period). If a subject admits a non-compliance with these restrictions, the Principal Investigator or attending Research Physician will decide whether or not the subject will be permitted to remain in the study. Non-compliance with these restrictions will be noted.

7.3. Other Life-Style Restrictions

7.3.1. Strenuous Exercise

Strenuous exercise must be avoided completely from 3 days before the first dose of IMP until the final study visit.

7.3.2. Blood Donation

Subjects will be advised that they should not donate blood for at least 3 months after the final study visit.

7.3.3. Contraception

To prevent pregnancy female subjects of childbearing potential and male subjects with female partners of child bearing potential must take adequate contraceptive precautions for the entire duration of study participation. Adequate contraceptive precautions include:

1. Established use of oral, injected or implanted hormonal methods of contraception.
2. Placement of an intrauterine device (IUD) or intrauterine system (IUS).
3. Barrier methods of contraception: Condom or occlusive cap (diaphragm or cervical/vault caps) with spermicidal foam/gel/film/cream/suppository.
4. Male sterilisation (with the appropriate post-vasectomy documentation of the absence of sperm in the ejaculate). *[For female subjects on the study, the vasectomised male partner should be the sole partner for that subject]*.

5. True abstinence, when this is in line with the preferred and usual lifestyle of the subject. [Periodic abstinence (e.g., calendar, ovulation, symptothermal, post-ovulation methods) and withdrawal are not acceptable methods of contraception].

The chosen contraception method(s) must be followed from the first dose until at least 3 months after the last dose of IMP.

7.3.4. Sperm Donation

Subjects must not donate sperm from the first dose and for at least 3 months after the last dose of IMP.

8. INVESTIGATIONAL MEDICAL PRODUCT (IMP)

8.1. Cytisine 1.5 mg Film-Coated Tablets

Cytisine will be supplied by the Sponsor. A Cytisine tablet is formulated as a compressed film-coated tablet containing 1.5 mg Cytisine in each single tablet (See Table 3).

Cytisine tablets are manufactured by [redacted] in accordance with cGMP. Each tablet is composed of cytisine (as the base), with well-established tablet-forming excipients [redacted]

Cytisine should be administered orally in a single dose application [redacted] taken with 240 mL water (8 fluid ounces).

Table 3: Cytisine Product Information

Name of the Product:	Cytisine 1.5 mg, film coated tablets [redacted]
Strengths:	[redacted]
Dosage form:	[redacted]
Route/mode of administration:	Oral
Dosing Schedule:	[redacted]
Dosing instructions:	[redacted]
Manufacturer/Marketing Authorization Holder:	[redacted]

The documentation supplied will make it possible to retrace the composition and pharmaceutical quality of the product.

8.2. Receipt and Storage

The test IMP will be supplied by the Sponsor as product in the commercial presentation.

The Sponsor must notify the Chief Investigator, or the Project Manager, prior to dispatch of the IMP supplies, and of the anticipated date of their arrival. IMP should arrive at the study site at least 7 days before the first dosing day. The Sponsor shall address all supplies to:

The IMP will be stored under quarantine in a segregated, study-specific area, at or below 25 ºC in a secure, temperature-controlled pharmacy. [redacted] Qualified Person (QP) will review the shipping documentation. If acceptable, the supplies will subsequently be removed from quarantine and approved for use.

8.3. Assembly and Certification

The IMP will be assembled into unit doses by suitably trained [redacted] staff according to SOP BD/324/13/01 and BD/324/13/25 as appropriate.

The IMP will be labelled as specified in Annex 13 (Manufacture of IMPs) of the European Commission (EC) guide to Good Manufacturing Practice (GMP).

Finished IMP will be certified by [redacted] Qualified Person according to SOP BD/324/13/29.

8.4. Administration

Each subject will receive IMP under both fed and fasted conditions over 2 treatment periods in accordance with the randomisation schedule, which will assign them to a scheduled period for either fed or fasting sequencing (Schedule A or Schedule B):

The dose to be administered is 3 mg Cytisine (2 x 1.5 mg tablets)

8.5. Return/Destruction

All used IMP containers and unused IMP will be held under quarantine in the [redacted] Pharmacy pending return/destruction. Following the Sponsor's approval, all remaining IMP will be destroyed or returned to the Sponsor within 8 weeks of the last dose of IMP.

8.6. Method of Assigning Subjects to Schedule A or Schedule B

Subjects will be randomized to treatment schedule according to a code produced by [redacted] using the PROC PLAN procedure of SAS® version 9.1.3 or higher. Subjects will be numbered sequentially from 001 (i.e. 001, 002 etc.). Replacement subjects will be assigned the same randomisation as the subject they are replacing, however, 100 will be added to the number (i.e., 101 would replace 001 etc.).

8.7. Selection of Doses in the Study

The dose selected for this study is 3 mg cytisine. The dose level was selected to allow adequate detection of cytisine in plasma samples for PK analysis.

8.8. Selection and Timing of Dose for Each Subject

Doses will be administered at approximately 9.00 a.m. All subjects will have been fasting for at least 10 h overnight prior to dosing. Subjects assigned to be fasting prior to dosing will remain fasting until lunch and subjects assigned to be fed prior to dosing will start their high-fat breakfast 30 minutes prior to the dose. Lunch will be provided 4 h after dosing for all subjects.

8.9. Blinding

The study is open-label. However, as stated in the EMA guidelines on investigation of bioavailability and bioequivalence (CPMP/EWP/QWP/1401/98 Rev.1/Corr** London, 20 January 2010), analysis of the samples should be conducted without information on treatment, therefore staff responsible for drug concentration and ECG analysis will be blinded to the treatment schedule.

9. PRIOR AND CONCOMITANT THERAPY

Prior Medication: Prescription or non-prescription drugs, including vitamins, herbal and dietary supplements should not be taken within 14 days (or 5 half-lives, whichever is longer) prior to the first dose of IMP, unless in the opinion of the Investigator and Sponsor's Responsible Physician the medication will not interfere with the study procedures or would compromise subject safety. Prescription or non-prescription drugs, including vitamins, herbal and dietary supplements taken during the 14 days before the first dose of IMP, and the reason for taking them, will be noted in the subject's CRF.

Inclusion of subjects who have taken prior medication will be reviewed on a case-by-case basis in relation to the safety aspects and objectives of this study.

Concomitant Medication: Prescription or non-prescription drugs, including vitamins, herbal and dietary supplements should not be taken throughout the duration of the study, with the exception of paracetamol (which may be taken as an analgesic to a maximum of 2 g in 24 h (500 mg 4 times a day) and ibuprofen (which may be taken as an analgesic to a maximum of 1.2 g in 24 h (400 mg 3 times a day).

If intake of ***ANY*** prior or concomitant medication is necessary during the study, the daily dosage, duration and reasons for administration will be recorded on the subject's CRF.

Chewing gum will not be allowed during confinement periods.

10. TREATMENT COMPLIANCE

Each dose of IMP will be taken under supervision and a hand and mouth check conducted. The exact dosing time for each subject will be recorded on the subject's CRF.

11. STUDY PROCEDURES

All subjects will be evaluated for inclusion in the study during the Screening Period. Subjects who are eligible for the study will be randomized according to the randomization schedule.

Subjects will receive their study medications on Day 1 of each period followed by the required blood and urine samples and ECG Holter monitoring. The subjects will be released from the clinical research facility after the last PK sample is collected and safety assessments completed in each period (approximately 24 hours post-last dose, mornings of Day 2 of each period). All subjects are required to complete a telephone follow-up on Day 6-8 days post last dose.

11.1. Procedure Schedule

Table 4 provides a summary of required study evaluations. Screening evaluations are to occur within a 28-day interval from initiation of screening evaluations to randomization on Day 1 of Period 1, pre-dose.

Table 4: Schedule of Study Procedures

Days	**Screening**	**Study Day (Period 1 and Period 2)**			**Post-Study Telephone Call**
Days	-28 to -2	Day -1	1	2	6-8 days after last dose
Written informed consent	X				
Demographic data/height/weight	X				
Randomization			X (Pre-first dose in Period 1)		
Vital signs	X	X	X[1]		
Medical history	X				
Medical history update		X[2]			
Prior and concomitant medication	X	X	X	X	
Physical examination	X				
12-lead ECG	X			X[3]	
Holter monitor for continuous ECG readings[4]		X	X	X	
Pregnancy test for all females	X (Serum)	X (Urine)			
FSH (females only)	X				
Haematology	X			X	
Biochemistry	X			X	
Urinalysis	X			X	
Drugs-of-abuse tests in urine	X	X			
Verification of eligibility criteria	X	X	X		
Cytisine administration			X		
Blood and urine collection for PK analysis			X	X	
Adverse events monitoring		X[5]	X[5]	X[5]	X[5]

[1] Vital signs (supine blood pressure, pulse rate and oral temperature) will be recorded at pre-dose and again approximately 2 hours after administration on Day 1 of each period.
[2] Clinically relevant changes will be reported as adverse events.
[3] Repeat 12-lead ECG and assess prior to discharge.
[4] Attach ECG Holter monitor and begin recording on Day -1 and continue until 24 hours post dose.
[5] Adverse event recording to begin upon admissions on Day -1 of Period 1 to post study.

11.2. Detailed Description of Study Visits

11.2.1. Screening Visit (Day -28 to Day -2)

All subjects will complete the following screening procedures within 28 days of Day 1 in order to verify required inclusion and exclusion criteria.

- Written informed consent
- Demographic data
- Medical history data
- Prior and concomitant medication
- Physical examination
- Vital signs
- 12-lead ECGs (triplicate)
- Clinical laboratory safety tests (biochemistry, haematology, urinalysis, virology, drugs of abuse and serum pregnancy test for females)
- Verification of eligibility criteria

11.2.2. Day -1 (Period 1 and Period 2): Admission

The results of screening for each subject must be reviewed by the Principal Investigator prior to subject's admission to assure eligibility criteria have been met.

On Day -1 of each period, the subject will check into the research facility and upon arrival to the research facility, the following procedures are to be performed:

1. Repeat urine testing for drugs of abuse and urine pregnancy test for female subjects.
2. Verify that all subjects still satisfy the inclusion and exclusion criteria.
3. Attach ECG Holter monitor, checking to ensure recording has started. Note: Holter monitors to remain attached to subjects until 24 hours post-dosing.

All subjects will fast for minimum of 10 hours prior to dosing on Day 1.

All subjects will remain in clinic overnight.

11.2.3. Day 1 (Period 1 and Period 2)

Administration of cytisine will occur on Day 1 of each period for all subjects.

The following study procedures are to be performed prior to dosing:

1. Check to ensure ECG Holter monitor is functioning properly after overnight monitoring.
2. Vital signs (supine blood pressure, pulse rate and oral temperature) will be recorded at pre-dose and again approximately 2 hours after administration.
3. Any clinically relevant changes should be documented as adverse events.
4. Prior and concomitant medication documented.

5. Pre-dose blood draw for PK to be taken within 60 minutes prior to dosing.

For Period 1 only: After Day 1 pre-dose study procedures have been successfully completed and all eligibility criteria has been verified, subjects will be randomized onto Schedule A or Schedule B.

Schedule A (12 subjects):

- Period 1: Cytisine (2 x 1.5 mg tablets) will be administered 30 minutes after the start of a high fat breakfast (fed state) with 240 mL (8 fluid ounces) water. No additional fluids are to be taken until 1 hour post-dosing
- Period 2: Cytisine (2 x 1.5 mg tablets) will be administered after the overnight fast of at least 10 hours and subject will continue fasting until lunch (fasting state). Dosing will be administered with 240 mL (8 fluid ounces) water. Except for fluids taken for dosing, no fluids will be allowed from 1 hour before dosing until 1 hour post-dosing.

Schedule B (12 subjects):

- Period 1: Cytisine (2 x 1.5 mg tablets) will be administered after the overnight fast of at least 10 hours and subject will continue fasting until lunch (fasting state). Dosing will be administered with 240 mL (8 fluid ounces) water. Except for fluids taken for dosing, no fluids will be allowed from 1 hour before dosing until 1 hour post-dosing.
- Period 2: Cytisine (2 x 1.5 mg tablets) will be administered 30 minutes after the start of a high fat breakfast (fed state) with 240 mL (8 fluid ounces) water. No additional fluids are to be taken until 1 hour post-dosing.

Blood and urine samples will be collected and processed as outlined in (Section 12.1) and (Section 12.2) prior to, and after administration of cytisine (time zero).

No food should be allowed for at least 4 hours post-dose for either arm until lunch is served for all subjects.

All subjects will remain in the clinic overnight.

11.2.4. Day 2 (Period 1 and Period 2)

Remaining blood and urine samples for pharmacokinetics will be collected (Section 12.1).

1. Check to ensure ECG Holter monitor is functioning properly after overnight monitoring.
2. Document concomitant medications and any adverse events.
3. At 24 hours post-dosing, collect blood for haematology and biochemistry and collect urine for urinalysis.
4. Just prior to discharge after 24 hours post-dosing, remove ECG Holter monitor.
5. Obtain 12-lead ECG (in triplicate) similar to what was done at screen and review prior to discharge.

Upon final review by the study physician, the subject will be released from the clinical research facility and will be scheduled for return on Day 7.

11.2.5. Days 6-8 post last dose: Post-Study Follow-up

A Post-Study Follow-up telephone call will occur 6-8 days after last dosing to document any changes in adverse event(s) observed at discharge as well as any new adverse events. If any new adverse events are recorded at this follow-up call, arrangements will be made with the subject to come into the clinic for assessment and then followed until outcome determined.

12. DRUG CONCENTRATION MEASUREMENTS

12.1. Pharmacokinetic Blood Sampling

17 venous blood samples (volume of 7.5 mL each, total volume of approximately 128 mL) will be collected into pre-cooled lithium heparin tubes during each period, according to the following schedule:

Table 5: Blood Sampling for Pharmacokinetic Assessments During each Period

Blood Sample Number	Time from Dosing
1	Pre-dose (within 60 minutes prior to dosing)
2	00:15
3	00:20
4	00:30
5	00:45
6	01:00
7	01:30
8	02:00
9	02:30
10	03:00
11	04:00
12	06:00
13	08:00
14	12:00
15	14:00
16	16:00
17	24:00

The pre-dose blood samples will be collected within 60 minutes prior to dosing. The post-dose blood samples will be collected within +/-1 minute from the scheduled sampling time. The clock time will be recorded and reported for all blood draws and all subjects. A deviation greater than +/- 2 minutes will be reported as a protocol deviation and its cause will be recorded.

In case blood sampling for pharmacokinetics and other procedures coincide in time, blood draws will have priority unless other procedures are necessary for assuring subject's safety.

Immediately upon sampling, the blood sample for pharmacokinetic analysis will be identified with a bar-coded label bearing details of study number, subject number, study phase/period, sampling time point, sample type and a unique 9-digit identification number. The samples will be logged and then centrifuged at 1500 x g, 4°C for 10 minutes. Two equal aliquots of plasma will be transferred to polypropylene tubes labeled identically to the original blood sample (except sample type being plasma instead of whole blood) and logged when stored at approximately -80°C, pending analysis.

12.2. Pharmacokinetic Urine Collection

Six urine samples will be collected at the following time points during each period:

- Pre-dose (The pre-dose urine sample will be collected within 30 minutes prior to dosing)
- 0-2 hour
- 2-4 hour
- 4-8 hour
- 8-12 hour
- 12-24 hour

The urine collection containers will be identified with a bar coded label bearing details of the study number, subject number, study period number, sampling time-point, sample type and a unique 9 digit identification number.

The urine collection containers will be kept chilled (at approximately 4°C) during collections. After the termination of each fractional collection the volume excreted will be recorded and 2 x 10mL aliquots retained for determination of cytisine concentrations. The aliquots will be labelled identically to the original collection container and will be stored at -20°C pending analysis.

12.3. Determination of Plasma and Urine Drug Concentrations

Cytisine concentrations (ng/mL) in plasma and urine will be determined using a validated liquid chromatography tandem mass spectrometry method and will be conducted in accordance with Good Laboratory Practice regulations and guidelines. Methods validation must be completed prior to start of the analysis.

The sample analysis will only begin after the last sample of a study subject is collected. All samples from each subject will be analyzed in the same analytical batch. In each run, standard

and quality control samples will be distributed throughout the batch containing study samples. Samples with concentrations above the ULOQ will be diluted and re-assayed.

Full details, including the lower (LLOQ) and upper (ULOQ) limits of quantification range, will be provided in a Bioanalytical Phase Plan written by [redacted]

Plasma and urine samples of withdrawn subjects should also be analysed for drug concentrations and pharmacokinetic parameters (providing withdrawal was not due to withdrawal of consent).

Unacceptable values due to analytical reasons will be handled in accordance with the Bioanalytical Phase Plan.

Details of the methodologies used and the results obtained, including details of re-measured samples, will be given in the Analytical Study Report (ASR).

12.4. Incurred Samples Reanalysis

In order to establish the reproducibility of the assay, 10% of the first 1000 samples and 5% of the number of samples exceeding 1000 samples will be reanalyzed.

Both the original and replicate values will be presented in the Analytical Study Report with the percent difference between the two values. The original value will be the one used for pharmacokinetic analysis.

13. SAFETY ASSESSMENTS

13.1. Subject's Safety

Only healthy subjects are eligible for the study. The healthy status will be determined by meeting the inclusion and exclusion criteria. Subjects' safety will be monitored during the study. Adverse events will be monitored and recorded throughout the study.

Prior to the first IMP dosing only, the Principal Investigator or Research Physician will assess the clinical significance of results of laboratory investigations for haematology and serum chemistry values outside the defined normal ranges, as provided by the laboratory. All changes from screening that are observed on Day 2 during the study and meet clinically-significant definitions for laboratory adverse events will be recorded.

In addition to the planned times, any safety procedures can be performed at any time considered necessary by the Principal Investigator or attending Research Physician.

On each cytisine dosing day, at least one physician (Principal Investigator or Research Physician) will physically remain at the clinical site for investigational product administration and until 4 hours post-dose, and will remain available on call at all times during the entire period of the study.

In case any clinically significant abnormality is observed at pre-dose, Principal Investigator or attending Research Physician will decide whether the subject will proceed to investigational product administration or will be withdrawn from the study.

Safety will be assessed by consideration of all adverse events reported by or elicited from the subject and abnormalities detected on haematology, serum chemistry tests, urinalysis, and 12-

lead ECG. Worsening of other preexisting medical conditions and any changes to concomitant medications/treatments will also be taken into account in this evaluation.

All adverse events (serious and non-serious) beginning on Day 1 (prior to dosing) through post-study follow-up telephone call will be recorded in the subject's CRF.

13.2. Adverse Events

13.2.1. Definition of Adverse Event

The definitions are adopted in accordance with the Directive 2001/20/EC, ICH-E2A and the European Commission Detailed Guidance 2011/C 172/01.

An **Adverse Event** (AE) is defined as any untoward medical occurrence in a clinical trial subject administered a medicinal product and which does not necessarily have a causal relationship with the treatment.

An AE can therefore be any unfavorable and unintended sign (including any clinical significant abnormal laboratory finding), symptom, or disease temporally associated with the use of an investigational medicinal product, whether or not considered related to the investigational medicinal product.

An **Adverse Drug Reaction** (ADR) means all untoward and unintended responses to a medicinal product related to any dose administered. The phrase 'response to a medicinal product' means that a causal relationship has at least a reasonable possibility, i.e. the relationship cannot be ruled out and is judged by the Investigator as at least possible (see definition below).

An **Unexpected Adverse Drug Reaction** (UADR)/**Unexpected Adverse Event** (UAE) means an adverse reaction/event, the nature or severity of which is not consistent with the applicable product information, namely in the Investigator Brochure for an unauthorized investigational product or in the SmPC for an authorized product.

The expected/unexpected status should be evaluated and assessed, by the Sponsor, based on the reference safety information available since expectedness in Pharmacovigilance refers strictly to the information listed or mentioned in the applicable reference safety information and not to events that might be anticipated from knowledge of the pharmacological properties of a substance or because it was foreseeable due to the health status (e.g., age, medical history) of the study subjects.

A **Serious Adverse Event** (SAE) or **Serious Adverse Reaction** (SAR) is defined as an AE that results in any of the following:

- Results in death
- Is life-threatening
- Requires hospitalization or prolongs existing inpatient's hospitalization
- Results in persistent or significant disability or incapacity
- Results in a congenital abnormality or birth defect
- Is an important medical event which requires medical intervention to prevent any of the above outcomes

SUSARs: AEs which meet all of the following criteria:

- Serious.
- Unexpected (i.e., is not consistent with the applicable product information e.g. Investigator's brochure for an unapproved IMP or SmPC for an authorised product).
- There is at least a reasonable possibility that there is a causal relationship between the event and the medicinal product.

will be classified as suspected unexpected serious adverse reactions (SUSARs).

Important medical events are those which may not be immediately life-threatening, but may jeopardize the subject and may require intervention to prevent one of the other serious outcomes listed above. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm, or blood dyscrasias or convulsions that do not result in hospitalization.

The term **"life-threatening"** in the definition of "serious" refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe. For example, drug-induced hepatitis that resolves without evidence of hepatic failure would not be considered life threatening even though drug-induced hepatitis can be fatal.

Inpatient **hospitalization** or prolongation of existing hospitalization means that hospital inpatient admission and/or prolongation of hospital stay were required for treatment of AE or occurred as a consequence of the event. It does not refer to pre-planned elective hospital admission for treatment of a pre-existing condition that has not significantly worsened, or to diagnostic procedures.

13.2.2. Recording of Adverse Events

All of the following details will be recorded in the subject's CRF for each AE:

- Full description of AE.
- Date and time of onset.
- Date and time of resolution.
- Severity of event, to be assessed by an Investigator in accordance with the definitions below.
- Relationship to IMP to be assessed by an Investigator in accordance with the definitions below.
- Action taken (if any).
- Outcome and details of any further follow-up.

Adverse events documented in the CRF without a stop date should be reviewed at the end of each treatment period until final resolution or until it is medically justifiable to stop further follow up (e.g. a chronic condition has been reached.) Documentation of adverse events should be updated as necessary.

13.2.3. Grades of Adverse Event Severity

The following grades will be used by an Investigator to describe the severity of all AEs (including clinically-significant laboratory AEs) as shown in Table 6. Only 1 severity grade will be used for each AE (e.g. mild - moderate is not acceptable).

Table 6: Adverse Event Severity

Severity of AE	Definition
Mild	No interference with activity
Moderate	Some interference with activity not requiring medical intervention
Severe	Prevents daily activity and requires medical intervention

If an adverse event has multiple aspects, the aspect in the highest intensity will be graded. It is emphasized that the term severe is a measure of intensity; thus a severe AE is not necessarily serious. For example, itching for several days may be rated as severe; however, may not be clinically serious.

13.2.4. Assessment of Causal Relationship

The causal relationship between an adverse event and **cytisine** will be determined and documented by the responsible Investigator, or designee, according to best medical judgment as shown in Table 7.

Table 7: Assessment of Causal Relationship to Cytisine	
Category	**Description**
Not Related	The event is definitely not associated with cytisine.
Unlikely	The event was most probably produced by other factors such as the subject's clinical state, therapeutic intervention or concomitant therapy, and does not follow a known response pattern to cytisine
Possible	The event follows a reasonable temporal sequence from the time of cytisine administration, and/or follows a known response pattern to the investigational product, but could have been produced by other factors such as the subject's clinical state, therapeutic intervention or concomitant therapy.
Probable	The event follows a reasonable temporal sequence from the time of cytisine administration, and/or follows a known response pattern to the product, and could not have been produced by other factors such as the subject's clinical state, therapeutic intervention or concomitant therapy.
Definite	The event follows a reasonable temporal sequence from the time of cytisine administration, and/or follows a known response pattern to the product, and could not have been produced by other factors such as the subject's clinical state, therapeutic intervention or concomitant therapy, and either occurs immediately following investigational product administration, or improves on stopping the investigational product.

13.2.5. Reporting of Serious Adverse Events

Any new SAE that occurs during a treatment period should be recorded and reported immediately. All SAEs including those that are ongoing at the end of study treatment will be followed until each event resolves or is assessed as chronic.

In order to satisfy regulatory requirements, any Serious Adverse Event, whether deemed study drug-related or not, must be reported to the Sponsor or designee as soon as possible after the Investigator or coordinator has become aware of its occurrence. SAE form completion and reporting must not be delayed even if all of the information is not available at the time of the initial contact.

SAEs must be reported within 24 h of knowledge of the event by submitting an initial SAE report via email or fax to [redacted] will notify the Sponsor and Sponsor's Responsible Physician of the SAE via email within 24 h of receipt of the initial SAE report.

Additional information (follow-up) about any SAE unavailable at the initial reporting should be forwarded by the site within 24 hours of the information becoming available.

The following information should be provided to accurately and completely record the event:

- Investigator name and center number
- Subject number
- Subject initials, if permitted
- Subject demographics
- Clinical event
  - description
  - date of onset
  - severity
  - treatment
  - relationship to study drug (causality)
  - action taken regarding study drug
- If the AE resulted in death:
  - cause of death (whether or not the death was related to study drug)
  - autopsy findings (if available)
- Medical history case report form (copy)
- Concomitant medication case report form (copy)

- Any relevant reports (laboratory, discharge, x-ray, etc.)

Subjects who have had an SAE during the treatment period must be followed clinically until all parameters (including laboratory) have either resolved or been assessed as chronic.

SUSARs should be reported to the REC, MHRA and Sponsor in accordance with applicable regulatory requirements for expedited reporting. It is [redacted] responsibility to report SUSARs to the REC and MHRA. It is the Sponsor's responsibility to report any SUSAR to the FDA.

13.2.6. Monitoring of Subjects with Adverse Events

In the event of any abnormalities considered to be clinically significant by the investigating physician, subjects will be followed up with appropriate medical management until:

- It has resolved/returned to normal or baseline.
- The event has stabilised at a level acceptable to the Investigator and is not considered to be clinically significant.

13.3. Pregnancy

Serum pregnancy test will be performed for all females regardless of post-menopausal or sterilised status at screening. Pregnancy tests will be performed by [redacted] Serum pregnancy tests will be performed using the Roche Cobas c6000 analyser series comprising of c501 and e601 modules.

Pregnancies must be reported immediately and followed up to determine outcome, including spontaneous or voluntary termination, details of the birth, and the presence or absence of any birth defects, congenital abnormalities, or maternal and/or new-born complications. Pregnancy outcomes must be collected for females who took the IMP and the female partners of any males who took the IMP. Consent to report information regarding these pregnancy outcomes should be obtained from the mother.

13.4. Laboratory

13.4.1. Routine Laboratory Assessments

Routine laboratory safety samples will be analysed by [redacted] at screening and again on Day 2 of treatment periods 1 and 2 for each subject. Printed laboratory reports will include normal reference ranges. A decision regarding whether the result outside the reference range is of clinical significance or not shall be made by an Investigator and the report will be annotated accordingly. Clinically significant abnormalities at screening or occurring during the study will be recorded on the AE page. The reference ranges for laboratory parameters will also be filed in the Investigator site file.

Haematology: Haemoglobin (HGB), haematocrit (HCT), mean cell volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC), red blood cells (RBC), white blood cells (WBC), neutrophils (NEUT), lymphocytes (LYMP), monocytes (MONO), eosinophils (EOS), basophils (BASO) and platelets (PLT).

Samples will be collected into a 2.7 mL ethylenediaminetetraacetic acid (EDTA) Sarstedt Monovette® collection tube and analysed using the Siemens Advia 2120® or Siemens Advia 120®.

Biochemistry: Total protein (TP), albumin (ALB), total bilirubin (BIL-T), alanine transaminase (ALT), aspartate transaminase (AST), gamma glutamyl transferase (GGT), alkaline phosphatase (ALKP), glucose (GLU), sodium (NA), potassium (K), calcium (CA), bicarbonate (HCO_3), creatinine (CREA) and urea.

At screening, biochemistry samples will be collected into a Sarstedt Monovette® 4.7 mL z-gel collection tube. This volume allows sufficient serum for virology and pregnancy test screening. At all other visits, biochemistry safety samples will be collected into a 4.5 mL plain Sarstedt Monovette® collection tube. Biochemistry samples will be analysed using the Roche Cobas c6000 analyser series comprising of c501 and e601 modules.

Urinalysis: pH, specific gravity, protein, blood, glucose, leukocyte esterase, ketones, urobilinogen, bilirubin & nitrites. In the event that the 'dipstick' is positive, RBCs, WBCs, epithelial cells, crystals, bacteria and casts will be examined microscopically**.** The microscopy will be performed at [redacted] or referred to a local specialist hospital laboratory for analysis.

A mid-stream urine sample will be collected into a 20 mL Sterilin tube. Urinalysis will be performed using the Siemens Clinitek 500 analyser.

13.4.2. Virology

Virology (Hep B, Hep C and HIV) will be performed at screening.

Virology will be analysed from the Sarstedt Monovette® 4.7 mL z-gel biochemistry screen by [redacted] using the Roche Cobas c6000 analyser series comprising of c501 and e601 modules.

13.4.3. Drugs of Abuse and Alcohol

Urine alcohol and drugs of abuse screen: Cannabinoids, amphetamines, cocaine, benzodiazepines, opiates and cotinine.

A mid-stream urine sample will be collected into a 20 mL Sterilin tube. At time-points when both urinalysis and drugs of abuse / alcohol screening is required, all testing will be performed from a single 20 mL sample.

Drug of abuse and alcohol samples will be analysed by [redacted] using the Roche Cobas c6000 analyser series comprising of c501 and e601 modules.Vital signs

13.5. Vital signs

Systolic/diastolic blood pressure and pulse rate and oral temperature.

Measurements will be recorded in the supine position after 5 min supine. Blood pressure, pulse and temperature will be measured by the DINAMAP* Compact Vital Signs Monitor (Model TS) or equivalent. Normal ranges for vital signs are presented in Appendix 1.

13.6. Physical Examination

A physical examination will be performed by an Investigator. The examination will include general appearance, head, ears, eyes, nose, throat, neck, skin, cardiovascular system, respiratory system, gastrointestinal system, central nervous system, lymph nodes and musculoskeletal. An Investigator can examine other body systems if required, at their discretion.

13.7. ECG Measurements

Screening and discharge ECGs will be conducted using a 12-lead ECG. Heart rate, PR interval, QRS width, QT interval, and QT interval corrected using Fridericia's formula (QTcF) will be documented.

12-lead ECG recordings will be made using a MAC 5500 or equivalent. Each ECG trace should be labelled with the study number, subject number, subject initials and date of birth. An Investigator will provide an interpretation of each tracing. Clinically significant abnormalities will be recorded on the AE page. Normal ranges for 12-lead ECG parameters are presented in Appendix 1.

13.8. 12-Lead ECG Holter

12-lead ECG Holter recording will begin on Day -1 and continue until approximately 24 hours post dosing during each study period.

13.9. Concomitant Medication

All prior and concomitant medications taken during the study will be recorded in the subject's CRF.

14. APPROPRIATENESS OF MEASUREMENTS

All measurements performed in the study are standard measurements.

The total volume of blood to be collected from each subject during the study (approximately 280 mL) is considered acceptable.

Table 8: Summary of Blood Volume

Procedure	Visit	No. Samples	Blood Volume per Sample (mL)	No. Treatment Periods	Blood Volume (mL)
Biochemistry	Screening[a] Study Day 2	1 2	4.7 4.5	2	4.7 9.0
Haematology	Screening Study Day 2	1 2	2.7 2.7	2	2.7 5.4
PK Drug Conc. Measurement	Treatment Period	17	7.5	2	255
				Total Blood Volume	276.8

[a] From the screening biochemistry blood samples, the serum pregnancy test and virology screen will be conducted.

15. STATISTICAL METHODS

15.1. Primary Objectives and Analysis

All data recorded during the course of the study or derived during the programming phase will be presented in individual data listings.

Unless specified otherwise, continuous variables will be summarised with the following descriptive statistics: n (number of observations), arithmetic mean, standard deviation (SD), minimum value, median and maximum values. For PK summaries, the geometric mean and coefficient of variation (%CV) will also be used to summarise the data. Categorical data will be summarised with frequencies and percentages.

Unless specified otherwise, the minimum and maximum statistics will be presented with the same precision as the original data. The mean, median and geometric mean will be presented to one more decimal place than the original data. The SD will be presented to two more decimal places than the original data. Unscheduled measurements will be listed in the individual data listings. With the exception of baseline, unscheduled measurements will be excluded from the descriptive statistical analysis. In case an unscheduled measurement is performed immediately after the scheduled measurement due to an error in the previous measurement, the unscheduled measurement will be included in the analysis and the original erroneous measurement will be excluded.

For subject characteristics and safety analyses, missing data will not be replaced; descriptive statistics and statistical analysis will be performed on the basis of the available data only. Subjects discontinued will be included in the descriptive statistics if they have received the investigational product and their examinations were performed at the same scheduled time as other subjects. All data recorded on discontinued subjects will be listed.

15.2. Subject Population for Analyses

15.2.1. Safety Analysis Set

All subjects who received at least one dose of cytisine will constitute the safety population.

15.2.2. PK Analysis Set

The PK Analysis Set will include all subjects who receive both doses of cytisine and comply with the following criteria:

- Do not have an occurrence of vomiting or diarrhoea which renders the concentration profile unreliable;
- Do not use a concomitant medication which renders the concentration profile unreliable;
- Do not have a pre-dose concentration that is greater than 5% of the corresponding C_{max};

- Have at least one evaluable concentration that is preceded by a lower evaluable concentration and followed by a lower evaluable concentration for the calculation of C_{max}, T_{max} and AUCs (i.e. at least 3 evaluable concentrations in total);
- Do not violate the protocol (major protocol violation) in a way that may invalidate or bias the results.

15.3. Subjects Characteristics

Separate summaries of demographics, will be provided for both safety and PK analysis sets if applicable.

The protocol violations will be listed per subject, describing the nature of the violation. Subjects failing to complete the study (as well as the date and reasons for discontinuation) will be displayed. Time may be added if relevant to the nature of the violation.

Medical history will be referred in accordance with the Medical Dictionary for Regulatory Activities (MedDRA), version 20.0 or higher. Medications will be mentioned according to the Anatomical Therapeutic Chemical (ATC) classification system using WHO Drug Dictionary.

15.4. Sample Size Calculation

A total of approximately 24 subjects will be randomized to the study, with approximately 12 subjects per treatment schedule arm.

Subject numbers are based on the following guidance: No previous pharmacokinetic studies with cytisine have reported the effects of repeated administration, so no estimates of intra-subject variability of C_{max} or AUC are available. A recent bioavailability study in a limited number of dogs,[14] where cytisine was given in the fed and fasted states, indicated an increased C_{max} in the presence of food of 28%. A previous study[13] reported a total coefficient of variation (%CV) for C_{max} of 42%. Under the assumptions that a similar food effect will be observed in humans and that the intra-subject %CV will be less than the total %CV obtained from the previous study, based on an estimated geometric mean ratio for C_{max} of 1.30 (30% increase) and an estimated %CV of 35%, a sample size of 24 should be sufficient to meet the objectives of the study.

Subjects who withdraw from study at any time after randomization will not be automatically replaced. Replacement based on the number and reason of withdrawals will be at discretion of the Sponsor, following discussion with the Principal Investigator.

15.5. Plasma and Urine Concentrations

Descriptive statistics to summarize plasma and urine concentrations at each time point. Concentrations below the LLOQ will be replaced by zero for calculation of descriptive statistics.

Individual drug plasma concentrations versus time profiles will be plotted per subject on both a linear and a semi-logarithmic scale. For plotting of individual data in linear scale, concentrations below the LLOQ will be replaced by zero. For plotting of individual data in semi-logarithmic scale, concentrations below the LLOQ will be set to missing. Graphical presentation of individual data will be based on actual blood sampling times.

Arithmetic mean drug plasma concentration versus time profiles will be presented on both a linear and a semi-logarithmic scale.

The post-dose blood samples will be collected within ±2 minutes from the scheduled sampling time. A deviation greater than ±2 minutes will be reported as a protocol deviation.

15.6. Estimation of pharmacokinetic parameters

Plasma: The following pharmacokinetic parameters for cytisine will be derived by standard non-compartmental methods from the concentration versus time profiles:

Table 8: Cytisine Pharmacokinetic Parameters

Parameter	Description
C_{max}	Maximum observed plasma concentration post dose, directly obtained from the observed concentration versus time profile.
T_{max}	Time of occurrence of maximum observed plasma concentration.
AUC_{0-t}	Area under the plasma concentration versus time curve (AUC) from time zero to the last sampling time at which concentrations were at or above the LLOQ, calculated by the linear-up/log-down trapezoidal rule.
$AUC_{0-\infty}$	Total AUC from time zero to infinity, calculated as $AUC_{0-\infty} = AUC_{0-t} + (C_{last}/\lambda_z)$, where C_{last} is the last measurable plasma concentration and λ_z the apparent terminal elimination rate constant.
%AUC	Residual area or percentage of extrapolated part for the calculation of $AUC_{0-\infty}$, calculated as $100*[1-(AUC_{0-t}/AUC_{0-\infty})]$
λ_z	Apparent terminal elimination rate constant.
$t_{1/2}$	Apparent terminal elimination half-life, calculated from $ln\ 2/\lambda_z$

No values of λz, AUC0-∞, %AUC and t1/2 will be reported for cases where λz cannot be reliably determined.

Pharmacokinetic parameters will be estimated from the plasma concentration versus time profiles for all subjects in the safety analysis set. Actual sampling times will be used for the pharmacokinetic analysis. All calculations will be performed using raw data.

The terminal elimination rate constant (λz) will be determined by plotting the concentration data versus time on a semi-logarithmic scale. The parameter will be estimated by linear least square regression analysis, using the last three (or more) non-zero concentrations. The upper and the lower timepoints, as well as the number of timepoints, used for λz estimation will be reported.

AUC0-t will be calculated by the linear-up/log-down trapezoidal method.

Individual pharmacokinetic parameters and descriptive statistics for the pharmacokinetic parameters obtained for each treatment will be presented by treatment and gender (males, females, overall) and by treatment and BMI. If no reliable pharmacokinetic parameter could be determined for more than 1/3 of the subjects, only the minimum and maximum values will be presented for that parameter and all other descriptive statistics will be omitted. Missing pharmacokinetic parameter data will not be imputed.

Urine: The urine PK parameters amount excreted in urine over time (Ae) and percent of drug excreted in urine (Ae%) will be derived from the urine concentration-time data for all subjects in the safety analysis set. Derived urine PK parameters will be listed and summarised by treatment and gender (males, females, overall).

15.7. Pharmacokinetic Analysis

For males, females and overall (data from both genders pooled) following logarithmic transformation of $C_{max,}$ AUC_{0-t} and AUC_{0-inf} values will be subjected to an analysis of variance (ANOVA) including fixed effects for sequence, period, treatment and subject nested within sequence. Point estimates and 90% confidence intervals (CI) will be constructed for the contrasts between treatments using the residual mean square error obtained from the ANOVA. The point and interval estimates will be back-transformed to give estimates of the ratios of the geometric least squares means (LSmeans) and corresponding 90% CI. Estimated geometric means will also be presented for each treatment.

In addition, for the purpose of exploring whether BMI may have an effect on bioavailability, the above analysis will be performed by BMI category (BMI of 18-22, 23-27 or 28-32 kg/m^2).

A comparison of T_{max} between treatments will be performed using the Wilcoxon Signed-Rank test. In addition, the Hodges-Lehmann estimate of the median difference in T_{max} and corresponding 95% CI will be presented. This analysis will be performed by gender and overall.

15.8. Safety Variables and Analyses

All safety data will be listed using the safety analysis set.

15.8.1. ECGs

The ECG extraction timepoints will be from extraction windows which precede the PK blood draws. The following ECG parameters will be extracted in triplicate from Holter recordings at 45, 30 and 15 minutes prior to pre-dose and at pre-dose, 0.5h, 1h, 2h, 3h, 4h, 6h, 8h, 12 h and 24h post dose (Period 1 and Period 2):

- Heart Rate (HR)
- PR intervals
- QRS
- QT
- QTcF

If a large change in heart rate is observed during the study, the individual QT correction method (QTcI) will be used as the primary QT correction method.

The extracted ECG parameters will be listed with any out of normal range values flagged (“H” - high or “L” -low). The ECG results will assessed as either “normal” or “abnormal”. Any comments on abnormal results will also be provided. Descriptive statistics of absolute and change from baseline values at each time point will be tabulated.

In addition, frequencies of QTcF data will be calculated according to the following categories:

For absolute values:

- QTcF ≤ 450 mSec
- 450 < QTcF ≤ 480 mSec
- 480 < QTcF ≤ 500 mSec
- QTcF > 500 mSec.

For change from baseline:

- QTcF increase ≤ 30 mSec
- 30 < QTcF increase ≤ 60 mSec
- QTcF increase > 60 mSec.

Abnormalities in ECGs will be classified in terms of clinical significance. Clinically significant abnormalities will be reported as AEs

In order to explore the effects of cytisine on QTc prolongation, plasma concentrations will be plotted against change from baseline QTcF values.

15.8.2. Vital Signs

Vital signs parameters will be listed with any out of normal range values flagged ("H" -high or "L" -low) as described in the Statistical Analysis Plan (SAP).

Abnormalities in vital signs will be classified in terms of clinical significance. Clinically significant abnormalities will be reported as AEs.

15.8.3. Adverse Events

AEs will be tabulated and summarised according to the MedDRA version 20.0 or higher, and classified by system organ class (SOC) and preferred term (PT). The following information recorded or computed is used for the description of the adverse events: reported Term; SOC and PT by MedDRA coding; onset date, onset time, offset date and offset time; seriousness; severity (intensity); relationship (causality); action taken; outcome.

Treatment-emergent adverse events (TEAEs) are defined as AEs not present prior to first administration of investigational product, or AEs present before first administration of investigational product that worsen after the subject receives the first dose of investigational product. TEAEs that occur after administration of investigational product in a given period are assigned to the treatment administered in that period. A separate listing of SAEs will be presented, if applicable.

Frequencies of TEAEs will be presented by SOC and PT, severity and relationship, by Fed/Fasting regimens and overall.

15.8.4. Safety Laboratory tests

Biochemistry, haematology and urinalysis parameters will be listed with any out of normal/alert range values flagged. Laboratory test results which are out of normal range will also be presented separately along with normal reference ranges.

Abnormalities in clinical laboratory tests will be classified in terms of clinical significance. Clinically significant abnormalities will be reported as AEs.

15.9. Procedures for Deviations from Planned Analyses

The planned statistical analyses for this study will be described in detail in a SAP. Modifications or additions to the analyses described above will be described in the SAP. Any decisions to deviate from the planned analyses described in the protocol and SAP will be documented in the clinical study report. The clinical study report will also provide a detailed explanation for any deviations from the planned analyses.

16. DOCUMENTATION

16.1. Study File and Site Documents

Prior to the initiation of the study, the following items must be received by the Sponsor from the site:

- Confidential Disclosure Agreement
- Signed protocol, and amendment(s) page(s)
- The Principal Investigator's curriculum vitae and where required current medical license
- Signed Clinical Study Agreement
- Signed Financial Disclosure Form from the relevant site personnel
- Signed Data Protection Form (for EU member states only)
- Competent/Regulatory Authority written approval (if applicable)
- EC written approval for the protocol, amendment(s), Informed Consent Form, Subject Information Sheet (if applicable), advertisements (if applicable)
- EC Membership list or an official statement from the EC/IRB stating the EC/IRB is in compliance with the EU Directive on Good Clinical Practice (GCP)
- Local laboratory certification and normal ranges

16.2. Study Documents Supplied by the Sponsor

The Sponsor will supply the Investigator with the following items:

- Current version of the Investigator's Brochure
- Current version of study protocol

16.3. Maintenance and Retention of Records

It is the responsibility of the Investigator to maintain a comprehensive and centralized filing system of all relevant documentation.

- Investigators will be instructed to retain all study records required by the Sponsor and regulatory authorities in a secure and safe facility with limited access for one of the following time periods based on notification from the Sponsor:
  - For a period of at least 2 years from the **last** marketing approval worldwide or for at least 15 years, whichever is the greater
  - Or a period of at least two years after discontinuation of clinical development of the investigational product as confirmed by the Sponsor
  - For a longer period if required by local regulations

The Investigator will be instructed to consult with the Sponsor before disposal of any study records and to provide written notification to the Sponsor of any change in the location, disposition, or custody of the study files.

17. ADMINISTRATIVE PROCEDURES

17.1. Sponsor Responsibilities

The study will be monitored by representatives of the Sponsor and/or designated contract research organizations (CROs). Routine monitoring visits will be conducted to:

a. Assure compliance with the study protocol.
b. Verify that the research facilities, including laboratories and equipment, are adequate to safely and properly conduct the study.
c. Verify that the investigational product is stored properly and under the proper conditions, is in sufficient supply, and that receipt, use, and return of investigational product at the study sites are controlled and documented adequately.
d. Verify that written informed consent was obtained before any protocol-specific screening procedures are performed solely for the purpose of determining eligibility for the clinical study and/or prior to the provision of study medication.
e. Review the subject CRFs and source documents to ensure that reported study data are accurate, complete, and verifiable from source documents.
f. Ensure that adequate records of clinical trial supplies are maintained.
g. Verify that the Investigator and study site personnel are adequately qualified throughout the study.
h. Verify that the safety information and amendments are submitted to the IRBs/ECs/REBs.

17.2. Investigator Responsibilities

All requested study data must be entered on the CRFs for the study. An explanation should be provided for all missing data. Correction of data on a CRF will be made with identification of the individual making the correction and date of the correction. Only individuals who are identified on the Delegation of Responsibility Form(s) may correct data on the CRF. For those subjects who withdraw before completion of their specified treatment regimen, all available efficacy and safety data must be entered in the CRF. The reason for withdrawal must be specified. Incomplete

or inconsistent data on the CRFs will result in data queries that will be returned to the Investigator for resolution.

The Investigator must maintain adequate and accurate source documents upon which CRFs for each subject are based. The source documents are to be separate and distinct from the CRFs, except for cases in which the Sponsor has predetermined that direct entry into specified pages of the subject's CRF is appropriate. The documents to be maintained must include, but are not limited to, detailed notes on:

a. The medical history prior to participation in the study
b. The basic identifying information, such as demographics, that link the subject's source documents with the CRFs
c. The results of all diagnostic tests performed, diagnoses made, therapy provided, and any other data on the condition of the subject
d. The subject's exposure to study treatment
e. All AEs
f. The subject's exposure to any concomitant therapy, including dates of administration
g. All relevant observations and data on the condition of the subject throughout the study
h. The oral and written communication with the subject regarding the study treatment, including the risks and benefits of the study. The date of informed consent must be recorded in the source documentation.

18. REGULATORY COMPLIANCE

Quality Assurance representatives from the Sponsor or their delegate, the MHRA, and all other regulatory agencies as required will be allowed to periodically visit the Investigators to discuss the conduct of the trial and, upon request, to inspect the records of the trial. These reviews are necessary to ensure that the study is conducted according to standards consistent with the ICH GCP Guideline.

The Investigator agrees to discuss and correct, if necessary, any problems or deficiencies that are found during the course of these reviews.

19. ETHICAL CONDUCT OF THE TRIAL / GOOD CLINICAL PRACTICE

This trial will be conducted in accordance with the Declaration of Helsinki, as well as the ICH Guidelines on GCP, the US Code of Federal Regulations, the Food and Drugs Act (Health Canada), and local requirements regarding IRB/EC/REB committee review, informed consent, and other statutes or regulations regarding the protection of the rights and welfare of human subjects participating in biomedical research. The Chief Investigator shall be responsible for ensuring that the clinical study is performed in accordance with the following:

- Declaration of Helsinki (Brazil, 2013).
- Association of the British Pharmaceutical Industry (ABPI) Guidelines for Phase 1 Trials (2012).

- International Conference on Harmonisation (ICH) Harmonised Tripartite Guideline for Good Clinical Practice (GCP).
- The Medicines for Human Use (Clinical Trials) Regulations 2004 (Statutory Instrument 2004 No. 1031) and subsequent amendments.
- Applicable local standard operating procedures (SOPs).

As this clinical study will be conducted in the United Kingdom, it has been registered in the EudraCT database and a Clinical Trials Authorisation (CTA) will be obtained from the Medicines and Healthcare products Regulatory Agency (MHRA) prior to the start of the study in accordance with Part 3, Regulation 12 of the United Kingdom (UK) Statutory Instrument. In addition, this study protocol will be included in an Investigational New Drug (IND) application with the US FDA for cytisine development.

20. PROTOCOL MODIFICATION/PREMATURE TERMINATION

All protocol amendments must be written and approved by the Sponsor. Each IRB, EC, or REB will review and approve amendments prior to their implementation in the study. IRB, EC, or REB approval need not be obtained prior to removal of an immediate hazard to subjects.

The Sponsor may terminate the protocol early if safety or other issues occur.

The study may be suspended or terminated by either the Principal Investigator or the Sponsor after mutual consultation at any time for scientific and safety reasons. Furthermore, the study may also be terminated prematurely by the Sponsor for important corporate reasons, or due to instruction of the Regulatory Authorities due to safety reasons.

Following a decision of temporary suspension or discontinuation, it is a responsibility of the Principal Investigator to inform the study subjects, Ethics Committee and Regulatory Agency, stating the reasons for premature termination. [redacted] shall be responsible for expedited reporting and/or notification to MHRA/EMA. The Sponsor shall be responsible for expediting reporting and/or notification to other regulatory authorities, as applicable.

21. POLICY FOR PUBLICATION AND DATA PRESENTATION

The Sponsor encourages the scientific publication of data from clinical research trials. However, Investigators may not present or publish partial or complete study results individually. The Principal Investigators and the Sponsor may propose appropriate scientific manuscripts or abstracts from the study data. Any manuscript or abstract proposed by the Investigators must be reviewed to ensure accuracy of data represented and commented upon in writing by the Sponsor prior to submission for publication. Investigators agree to consider the comments of the Sponsor in good faith and the Sponsor agrees in good faith not to impose limitations on access to the complete study data or unreasonable or inappropriate restrictions on publication of the study results. In case of publication, confidentiality of the study volunteers will be maintained.

INVESTIGATOR'S AGREEMENT

Protocol No. ACH-CYT-01

I have carefully read the foregoing protocol including all appendices and agree that it contains all the necessary information for conducting the study safely.

I will conduct this study in strict accordance with this protocol and according to the current GCP guidelines and will attempt to complete the study within the time designated.

I will provide copies of the protocol and all other information submitted by the Sponsor relating to pre-clinical and prior clinical experience to all personnel for whom I am responsible that participate in the study. I will discuss this information with them to assure that they are adequately informed regarding the drug and conduct of the study.

I agree to keep records on all subject information (case report forms, shipment and drug return forms and all other information collected during the study) in accordance with the current GCP and local regulations.

Site Principal Investigator's name	Sponsor's Representative's name
Signature	Signature
Date **(ddMmmYYYY)**	Date **(ddMmmYYYY)**
Institution	

REFERENCES

1. WHO. WHO Report on the Global Tobacco Epidemic, 2008 The MPOWER package. Geneva2008.
2. Hughes JR, Keely J, Naud S. Shape of the relapse curve and long-term abstinence among untreated smokers. Addiction (Abingdon, England) 2004;99:29-38.
3. Doll R, Peto R, Boreham J, Sutherland I. Mortality in relation to smoking: 50 years' observations on male British doctors. BMJ (Clinical research ed) 2004;328:1519.
4. WHO. WHO Framework Convention on Tobacco Control2003.
5. Aveyard P, West R. Cytisine and the failure to market and regulate for human health. Thorax 2013;68:989.
6. Prochaska JJ, Das S, Benowitz NL. Cytisine, the world's oldest smoking cessation aid. BMJ (Clinical research ed) 2013;347:f5198.
7. Stapleton JA. The case for licensing cytosine now for smoking cessation is overwhelming. BMJ (Clinical research ed) 2013;347:f5736.
8. Gopalakrishnan M, Monteggia LM, Anderson DJ, et al. Stable expression, pharmacologic properties and regulation of the human neuronal nicotinic acetylcholine alpha 4 beta 2 receptor. The Journal of pharmacology and experimental therapeutics 1996;276:289-97.
9. Tutka P, Zatonski W. Cytisine for the treatment of nicotine addiction: from a molecule to therapeutic efficacy. Pharmacological reports : PR 2006;58:777-98.
10. Etter JF, Lukas RJ, Benowitz NL, West R, Dresler CM. Cytisine for smoking cessation: a research agenda. Drug and alcohol dependence 2008;92:3-8.
11. Gotti C, Guiducci S, Tedesco V, et al. Nicotinic acetylcholine receptors in the mesolimbic pathway: primary role of ventral tegmental area alpha6beta2* receptors in mediating systemic nicotine effects on dopamine release, locomotion, and reinforcement. The Journal of neuroscience : the official journal of the Society for Neuroscience 2010;30:5311-25.
12. Hughes JR, Stead LF, Hartmann-Boyce J, Cahill K, Lancaster T. Antidepressants for smoking cessation. The Cochrane database of systematic reviews 2014:Cd000031.
13. Jeong SH, Newcombe D, Sheridan J, Tingle M. Pharmacokinetics of cytisine, an alpha4 beta2 nicotinic receptor partial agonist, in healthy smokers following a single dose. Drug testing and analysis 2015;7:475-82.
14. SRI Study No. M2161-16.

APPENDIX 1. NORMAL RANGES FOR VITAL SIGNS AND ECG PARAMETERS

[redacted]

Normal Ranges for Vital Signs and ECG Parameters

VITAL SIGNS

Parameter	Normal Range	Units
Pulse rate	40 - 110	Beats per minute (bpm)
Systolic Blood Pressure	90 - 150	mmHg
Diastolic Blood Pressure	50 - 90	mmHg
Respiratory Rate	12 - 18	breaths per minute
Oral Temperature	35.0 – 37.5	degrees Celsius
Pulse Oximetry	94 - 100%	

ECG PARAMETERS

Parameter	Normal Range	Units
Heart Rate	40 - 110	Beats per minute (bpm)
PR interval	120 - 220	msec
QRS width	70 - 120	msec
QT interval	not applicable, should be corrected for heart rate	
QTc interval (= QTcB)	350 - 430 (males)	msec
	350 - 450 (females)	msec

SIGNED: [redacted] DATE: 19th March 2015

SIGNED: [redacted] DATE: 26 March 2015