

**Protocol ACH-CYT-02**

**Repeat-Dose Pharmacokinetic and Pharmacodynamic  
Evaluation of Cytisine in Healthy Smokers**

**September 21, 2017**  
**Version 2.0**

**CONFIDENTIAL**



**Achieve Life Sciences, Inc.**  
**19820 North Creek Parkway, Suite 201**  
**Bothell, Washington, U.S.A., 98011**  
**Tel: 425.686.1500 Fax: 425.686.1600**

**Achieve Life Sciences Technologies, Inc.**  
**1001 W. Broadway, Suite 400**  
**Vancouver, British Columbia, Canada, V6H 4B1**  
**Tel: 604.736.3678 Fax: 604.736.3687**

---

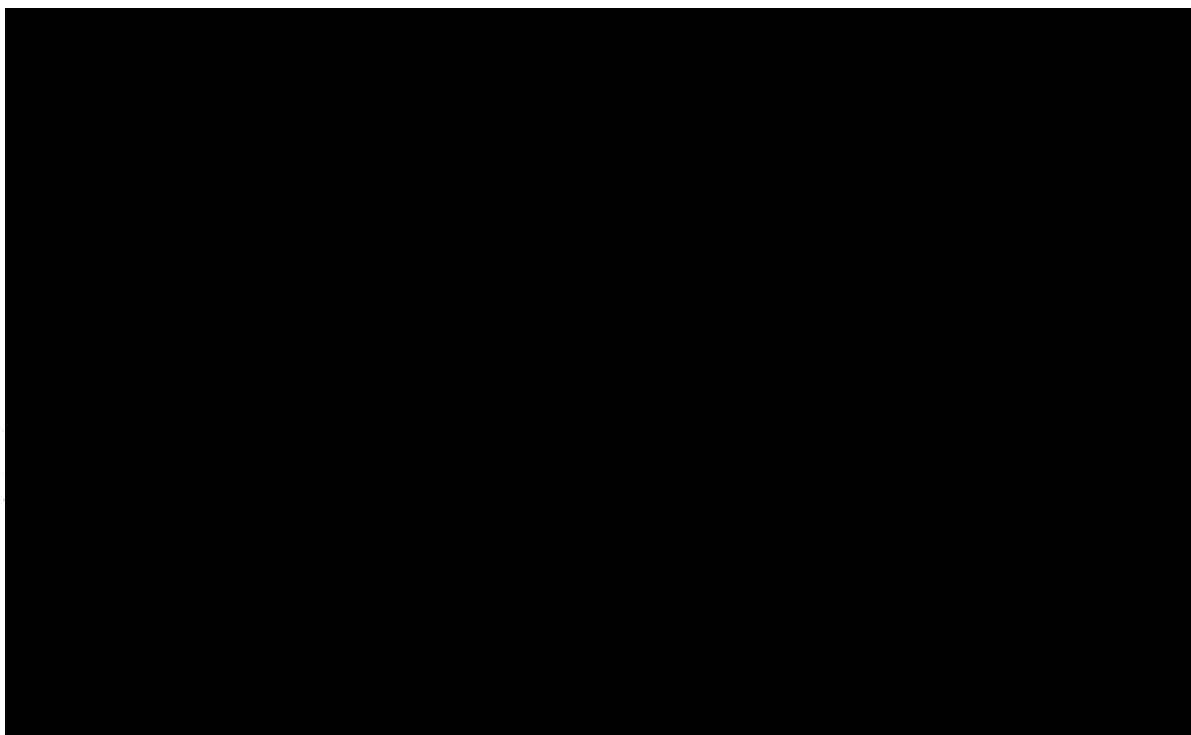
**SPONSOR SIGNATURE PAGE**

---

<b>PROTOCOL NUMBER</b>	ACH-CYT-02
<b>PROTOCOL TITLE</b>	Repeat-Dose Pharmacokinetic and Pharmacodynamic Evaluation of Cytisine in Healthy Smokers
<b>EudraCT NUMBER:</b>	2017-003257-42
<b>PLANNED STUDY DOSES:</b>	Mutiple doses of 1.5 mg cytisine and 3.0 mg cytisine
<b>PROTOCOL VERSION</b>	Version 2.0
<b>PROTOCOL DATE</b>	21 September 2017

---

Approvals:



## SYNOPSIS

<b>NAME OF COMPANY:</b> Achieve Life Sciences, Inc.
<b>NAME OF INVESTIGATIONAL MEDICINAL PRODUCT (IMP):</b> Cytisine 1.5 mg film-coated tablets
<b>TITLE OF STUDY:</b> Repeat-Dose Pharmacokinetic and Pharmacodynamic Evaluation of Cytisine in Healthy Smokers
<b>PRINCIPAL INVESTIGATOR:</b> [REDACTED]
<b>STUDY CENTRE:</b> Simbec Research Ltd (Simbec) Merthyr Tydfil, CF48 4DR, UK
<b>CLINICAL PHASE:</b> 1/2
<b>OBJECTIVES:</b> <i>Primary:</i> <ul style="list-style-type: none"><li>• To evaluate the pharmacokinetic (PK) parameters during repeat dosing of 1.5 mg or 3.0 mg cytisine when administered as the commercial 25-day schedule.</li><li>• To evaluate the pharmacodynamic (PD) effects (e.g. reduction in smoking) with repeat dosing of 1.5 mg or 3.0 mg cytisine when administered as the commercial 25-day schedule.</li></ul> <i>Secondary:</i> <ul style="list-style-type: none"><li>• To compare the PK parameters and tolerability for repeat dosing of 1.5 and 3.0 mg cytisine during the 25-day schedule in healthy smokers at 18-65 and &gt;65 (elderly) years of age.</li><li>• To assess the renal elimination of cytisine via measurement of urinary concentrations of cytisine during treatment on Day 1 and Day 25.</li><li>• To evaluate for effects on QT/QTc interval prolongation and cardiac safety during treatment on Day 1 and Day 25 in healthy smokers at 18-65 and &gt;65 (elderly) years of age.</li></ul>
<b>METHODOLOGY:</b> This is an open-label, randomised, multi-dose study to evaluate the PK profile and PD effect of cytisine when administered at doses of 1.5 mg and 3.0 mg following the commercialised 25-day schedule.
<b>NUMBER OF SUBJECTS:</b> A total of 36 subjects, such that 24 subjects are 18-65 years and 12 subjects are >65 years of age, will be randomised/enrolled to complete the study. An attempt will be made for 50% male and 50% female enrollment.
<b>MAIN INCLUSION CRITERIA:</b> Male or female smokers (minimum 10 cigarettes per day) aged 18 – 65+ years, with no allergy or

sensitivity to cytisine or any of its excipients, who are deemed healthy by meeting enrollment criteria and, unless surgically sterile, are willing to abstain from sexual intercourse or willing to use an effective method of contraception from the first dose and for 3 months after the last dose of cytisine (referred to as the IMP).

**IMP ADMINISTRATION:**

Each subject will receive the IMP (cytisine in the form of the commercial product [REDACTED], (1.5 mg or 3.0 mg dose cytisine) over a 25-day period according to the current [REDACTED] SmPC as follows:

Days	Regimen	Total Daily Dose		Approximate Interval
		1.5 mg Dose	3.0 mg Dose	
1-3	6 times daily	9.0 mg	18.0 mg	2 hours
4-12	5 times daily	7.5 mg	15.0 mg	2.5 hours
13-16	4 times daily	6.0 mg	12.0 mg	3 hours
17-20	3 times daily	4.5 mg	9.0 mg	4-5 hours
21-24	2 times daily	3.0 mg	6.0 mg	6 hours
25	Once daily	1.5 mg	3.0 mg	-

This schedule will require a total of 9 overnight stays (evenings of Day -1, Days 1, 2, 3 and Days 12, 16, 20, 24, and Day 25).

**CRITERIA FOR EVALUATION:**

**PK:**

***PK Endpoints:***

*Plasma:*

Estimated  $C_{max}$  values will be determined as follows:

- On Day 1, an estimate of  $C_{max}$  will be made after the first daily (morning) dose from venous blood samples collected immediately post dose at 00:30, 00:45, 01:00, 01:15, 01:30, and 02:00 hours. For the last evening dose on Day 1, Day 2, and Day 3, an estimate of  $C_{max}$  will be made from venous blood samples collected immediately post dose at 00:30, 01:00, 01:30, 02:00, 02:30, 03:00, 03:30, 04:00, and 05:00 hours.
- On Day 12, 16, 20, and 24, an estimate of  $C_{max}$  will be made after the last daily (evening) dose from venous blood samples collected immediately post dose at 01:00, 01:30, 02:00, 02:30, 03:00, 03:30 and 04:00 hours.

Estimated  $C_{min}$  values will be made from venous blood samples collected prior to the first morning dose on Day 4, Day 13, Day 17, Day 21 and Day 25.

On Day 25  $C_{max}$ ,  $T_{max}$ ,  $t_{1/2}$  and AUC will be determined after the administration of the final dose of cytisine from venous blood samples collected at the following timepoints: 00:30, 00:45, 01:00, 01:15, 01:30, 01:45, 02:00, 02:15, 02:30, 02:45, 03:00, 03:15, 03:30, 03:45, 04:00, 05:00, 06:00, 08:00, 10:00, 12:00 and 24:00 hours post-dose.

Total volume of blood drawn for the pharmacokinetic and clinical laboratory safety assessments will be up to approximately 328 mL per subject over a 26-day period.

*Urine:* Amount excreted in urine over 24 hours ( $A_e$ ) and percent of drug excreted in urine ( $A_e\%$ ) over 24 hours will be calculated on Day 1 and Day 25 of treatment.

**Safety:**

**Safety Endpoints:** The number of subjects with adverse events, serious adverse events, and adverse events leading to discontinuation will be documented. Adverse events will be assessed by clinical observation and spontaneous reporting by subjects. In addition, number of subjects with clinically significant abnormalities in 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 parameters (haematology, biochemistry and urinalysis) will be evaluated.

**STATISTICAL METHODS:**

All statistical analysis will be performed using SAS<sup>®</sup> version 9.3 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 dose group, age group and overall. Subject disposition and analysis sets (safety, PK, and PD) will also be listed and summarised by frequency.

**PK:** Pharmacokinetic parameters of cytisine will be derived from the plasma concentration-time profiles, by using a non-compartmental approach with a ln-linear terminal phase assumption. Actual times of sampling will be used.

The following pharmacokinetic parameters will be estimated: maximum observed plasma concentration ( $C_{max}$ ); minimum observed plasma concentration ( $C_{min}$ ); time of occurrence of  $C_{max}$  ( $T_{max}$ ); area under the plasma concentration versus time curve (AUC) from time zero (pre-dose) to the last sampling time with quantifiable concentrations ( $AUC_{0-t}$ ); AUC from time zero to infinity ( $AUC_{0-\infty}$ ); residual area or percentage of extrapolated part of  $AUC_{0-\infty}$  (%AUC); apparent terminal elimination rate constant ( $\lambda_z$ ); and apparent elimination terminal half-life ( $t_{1/2}$ ). In addition, the amount of cytisine excreted in urine over time ( $A_e$ ) and percent of drug excreted in urine ( $A_e\%$ ) will be derived from Day 1 and Day 25 24-hour urine collections.

**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 after the first dose of cytisine on Day 1 and Day 25 will be obtained from the Holter recordings will be listed and analysed.

**DURATION OF STUDY:**

Approximately 9 weeks for each individual (from screening to post-study follow-up).

## TABLE OF CONTENTS

SYNOPSIS .....	3
LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS .....	12
1. ETHICS .....	14
1.1. Research Ethics Committee .....	14
1.2. Subject Information and Consent .....	14
1.3. Indemnity Arrangements .....	15
2. INTRODUCTION AND BACKGROUND .....	15
2.1. Smoking and Health .....	15
2.2. Cytisine .....	15
2.3. Recent Cytisine Pharmacokinetic Studies .....	16
2.3.1. Study CYT-BIO-02-07 in Healthy Subjects .....	16
2.3.2. PK Study in Healthy Smoking Subjects published by the University of Auckland .....	18
2.4. Safety Summary .....	20
2.5. Recent Nonclinical Toxicology Studies: Estimation for Higher Human Equivalent Doses .....	20
3. RATIONALE FOR THE STUDY .....	22
4. STUDY OBJECTIVES .....	22
4.1. Primary Objectives .....	22
4.2. Secondary Objectives .....	22
5. INVESTIGATIONAL PLAN .....	22
5.1. Study Design .....	22
6. SELECTION OF STUDY POPULATION .....	25
6.1. Inclusion Criteria .....	25
6.1.1. To be Confirmed at Screening .....	25
6.1.2. To be Re-Confirmed Prior to Start of Dosing (Day -1) .....	27
6.2. Exclusion Criteria .....	27
6.2.1. To be Confirmed at Screening .....	27
6.2.2. To be Re-Confirmed Prior to Start of Dosing (Day -1): .....	27
6.3. Removal of Subjects from Therapy or Assessment .....	28
7. RETRICTIONS PRIOR AND DURING THE STUDY .....	28

7.1.	Confinement .....	29
7.2.	Smoking, Diet and Fluid Restrictions.....	29
7.2.1.	Smoking.....	29
7.2.2.	Meal Times .....	29
7.2.3.	Fluid Intake .....	29
7.2.4.	Alcohol Intake .....	29
7.2.5.	Caffeine.....	29
7.2.6.	Poppy and Sesame Seeds.....	29
7.2.7.	Grapefruit Juice and Other Restrictions .....	30
7.3.	Other Life-Style Restrictions.....	30
7.3.1.	Blood Donation.....	30
7.3.2.	Contraception.....	30
7.3.3.	Sperm Donation.....	30
8.	INVESTIGATIONAL MEDICINAL PRODUCT (IMP) .....	31
8.1.	Cytisine 1.5 mg Film-Coated Tablets .....	31
8.2.	Receipt and Storage .....	31
8.3.	Assembly and Certification .....	32
8.4.	Administration .....	32
8.5.	Return/Destruction.....	32
8.6.	Method of Assigning Subjects to Group A or Group B .....	32
8.7.	Selection of Doses in the Study.....	32
8.8.	Selection and Timing of Dose for Each Subject.....	32
8.9.	Dose Modifications for 3.0 mg Dose or Elderly Subjects (>65).....	33
8.10.	Blinding .....	34
9.	PRIOR AND CONCOMITANT THERAPY .....	34
10.	TREATMENT COMPLIANCE .....	35
11.	STUDY PROCEDURES .....	35
11.1.	Procedure Schedule .....	36
11.2.	Detailed Description of Study Visits .....	38
11.2.1.	Screening Visit (Day -28 to Day -2).....	38
11.2.2.	Day -1 .....	38
11.2.3.	Day 1 to Morning of Day 4.....	39
11.2.4.	Day 4 through Day 11.....	40

11.2.5.	Day 12 to Morning of Day 13.....	41
11.2.6.	Day 13 through Day 15.....	41
11.2.7.	Day 16 through Morning of Day 17 .....	42
11.2.8.	Day 17 through Day 19.....	42
11.2.9.	Day 20 through Morning of Day 21 .....	43
11.2.10.	Day 21 through Day 23.....	43
11.2.11.	Day 24 through Day 26.....	44
11.2.12.	Post Study Follow-up .....	45
12.	DRUG CONCENTRATION MEASUREMENTS .....	45
12.1.	Pharmacokinetic Blood Sampling .....	45
12.2.	Pharmacokinetic Urine Collection.....	49
12.3.	Determination of Plasma and Urine Drug Concentrations .....	49
12.4.	Incurred Samples Reanalysis.....	49
13.	SAFETY ASSESSMENTS .....	50
13.1.	Subject’s Safety .....	50
13.2.	Adverse Events .....	50
13.2.1.	Definition of Adverse Event.....	50
13.2.2.	Recording of Adverse Events .....	52
13.2.3.	Grades of Adverse Event Severity.....	52
13.2.4.	Assessment of Causal Relationship.....	53
13.2.5.	Reporting of Serious Adverse Events.....	53
13.2.6.	Monitoring of Subjects with Adverse Events .....	54
13.3.	Pregnancy .....	55
13.4.	Laboratory.....	55
13.4.1.	Routine Laboratory Assessments .....	55
13.4.2.	Expired Air Carbon Monoxide (CO).....	56
13.4.3.	Virology.....	56
13.4.4.	Drugs of Abuse and Alcohol .....	56
13.5.	Vital signs .....	56
13.6.	Physical Examination .....	56
13.7.	ECG Measurements.....	56
13.8.	12-Lead ECG Holter.....	57
13.9.	Subject Diary .....	57



13.10.	Concomitant Medication .....	57
14.	APPROPRIATENESS OF MEASUREMENTS .....	57
15.	STATISTICAL METHODS.....	58
15.1.	Primary Objectives and Analysis.....	58
15.2.	Subject Population for Analyses.....	58
15.2.1.	Safety Analysis Set .....	58
15.2.2.	PK Analysis Set .....	58
15.2.3.	Pharmacodynamic (PD) Analysis Set.....	59
15.3.	Subjects Characteristics .....	59
15.4.	Sample Size Calculation .....	59
15.5.	Plasma and Urine Concentrations.....	59
15.6.	Estimation of pharmacokinetic parameters .....	60
15.7.	Pharmacokinetic Analysis .....	60
15.8.	Pharmacodynamic Data .....	61
15.9.	Safety Variables and Analyses .....	61
15.9.1.	Holter ECGs.....	61
15.9.2.	12-Lead ECGs .....	62
15.9.3.	Vital Signs .....	62
15.9.4.	Adverse Events .....	62
15.9.5.	Safety Laboratory tests .....	63
15.10.	Procedures for Deviations from Planned Analyses .....	63
16.	DOCUMENTATION .....	63
16.1.	Study File and Site Documents.....	63
16.2.	Study Documents Supplied by the Sponsor.....	64
16.3.	Maintenance and Retention of Records.....	64
17.	ADMINISTRATIVE PROCEDURES .....	64
17.1.	Sponsor Responsibilities.....	64
17.2.	Investigator Responsibilities.....	65
18.	REGULATORY COMPLIANCE .....	65
19.	ETHICAL CONDUCT OF THE TRIAL / GOOD CLINICAL PRACTICE.....	66
20.	PROTOCOL MODIFICATION/PREMATURE TERMINATION.....	66
21.	POLICY FOR PUBLICATION AND DATA PRESENTATION .....	67
22.	INVESTIGATOR’S AGREEMENT .....	68

23.	REFERENCES .....	69
APPENDIX 1.	NORMAL RANGES FOR VITAL SIGNS AND ECG PARAMETERS .....	70
APPENDIX 2.	FAGERSTRÖM NICOTINE DEPENDENCE QUESTIONNAIRE .....	71
APPENDIX 3.	SHORT FORM OF THE TOBACCO CRAVING QUESTIONNAIRE .....	72

## LIST OF TABLES

Table 1:	Means of Pharmacokinetics Parameters for Cytisine .....	17
Table 2:	Mean Cytisine Urine Concentrations and Pharmacokinetic Parameters. ....	18
Table 3:	Calculation of Margin of Safety based on Rat and Dog Toxicology Studies.....	21
Table 4:	Cytisine Product Information .....	31
Table 5:	Dose Modification for 3.0 mg Dose Group .....	34
Table 6:	Schedule of Study Procedures .....	36
Table 7:	Blood Sampling for Pharmacokinetic Assessments .....	45
Table 8:	Adverse Event Severity .....	52
Table 9:	Assessment of Causal Relationship to Cytisine.....	53
Table 10:	Summary of Blood Volumes .....	57
Table 11:	Cytisine Pharmacokinetic Parameters .....	60

## LIST OF FIGURES

Figure 1:	Marketed Dosing (1.5 mg tablet) and Timing schedule for [REDACTED] .....	16
Figure 2:	Mean plasma concentrations (ng/mL) of cytisine over 24 hours .....	19
Figure 3:	Study Design Overview .....	23
Figure 4:	Dose Timing and Total Daily Dose .....	23

## LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

ABBREVIATION/TERM	DEFINITION
ADR	Adverse Drug Reaction
AE	Adverse Event
Ae	Amount excreted
ANOVA	Analysis of Variance
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
AUC <sub>0-t</sub>	AUC from Time Zero to Last Sampling Time with Quantifiable Concentrations
AUC <sub>0-∞</sub>	AUC from Time Zero to Infinity
%AUC	Residual Area or Percentage of Extrapolated Part of AUC <sub>0-∞</sub>
BMI	Body Mass Index
C <sub>last</sub>	Last Quantifiable Concentration
C <sub>max</sub>	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
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
MHRA	Healthcare products Regulatory Agency
NCCIH	National Center for Complementary and Integrative Health (branch of US NIH)
PD	Pharmacodynamic
PK	Pharmacokinetics
RBC	Red Blood Cell Count
REC	Research Ethics Committee
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SD	Standard Deviation
SmPC	Summary of Product Characteristics
SOC	(MedDRA) System Organ Class
SAP	Statistical Analysis Plan
SUSAR	Suspected Unexpected Serious Adverse Reactions
TEAE	Treatment emergent Adverse Events

---

<b>ABBREVIATION/TERM</b>	<b>DEFINITION</b>
$T_{\max}$	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
$\lambda_z$	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 may be no benefits gained by their participation. All potential conflicts of interest will be declared by the Principal Investigator and designees.

### **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. 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 Principal Investigator or designee 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. 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 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.<sup>1</sup> Smoking is highly addictive with more than 95% of unaided attempts at cessation failing to last 6 months.<sup>2</sup> Every year that a smoker delays quitting beyond the mid-30s, it has been estimated that the person loses 3 months of life expectancy.<sup>3</sup> 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.<sup>4</sup>

### 2.2. Cytisine

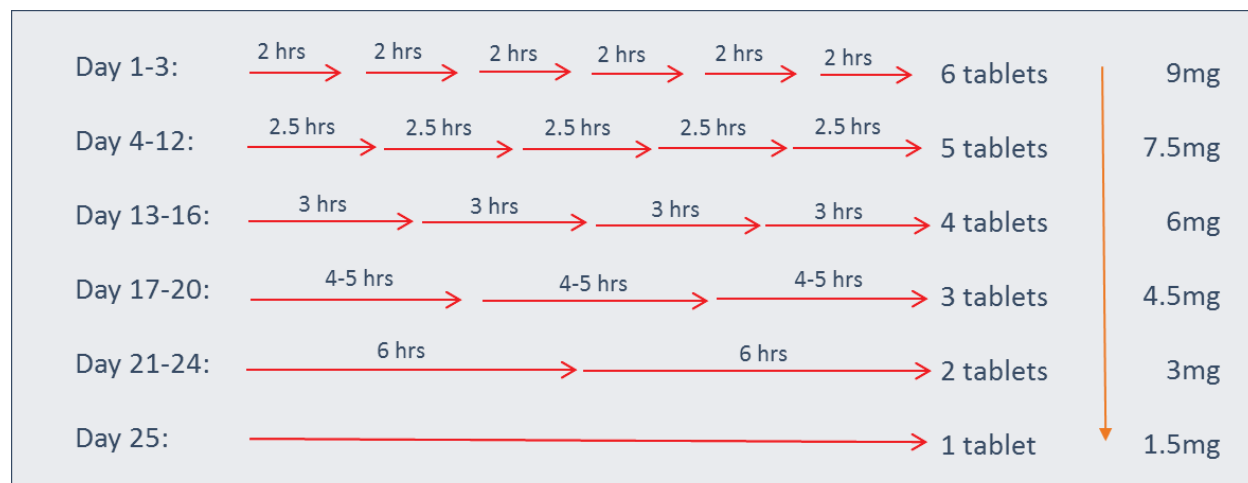
(-)-Cytisine, a plant-based alkaloid isolated from seeds of *Cytisus laburnum* (Golden rain acacia), has been used as a smoking cessation drug since the 1960's in Eastern and Central Europe, marketed as [REDACTED]. Despite its widespread use, cytisine has not been clinically developed outside of [REDACTED] Eastern and Central European territories. Achieve Life Sciences has acquired the license to cytisine outside of [REDACTED] territories to pursue clinical development and market approvals. There is widespread support for this strategy from leaders in the field.<sup>7-9</sup>

[REDACTED] focus on clinical development of cytisine was in its traditional markets in Eastern and Central European countries where the initial clinical studies were conducted and published, but not in English.<sup>5</sup> Because most of the clinical development trials leading to market approval in these countries occurred decades ago, the trials were not performed according to today's standard Good Clinical Practice (GCP) guidelines. Cytisine has been more recently evaluated in newer clinical trials, including additional pharmacokinetic (PK) studies and two large randomised Phase 3 clinical trials that were conducted according to GCP with more than 2,000 participants. The overall objectives in these more recent trials were to confirm the PK, efficacy, and safety of cytisine according to current clinical development standards. This clinical trial is being conducted to assess the pharmacokinetic parameters in far greater detail than has been reported for the commercial dose schedule as well as assessing a higher dose, as recommended by FDA for a future Phase 3 trial.

As a smoking cessation treatment using cytisine, the designated number of 1.5 mg cytisine tablets to achieve a particular dose or schedule are taken orally with approximately 240 mL of

water. The marketed dosing (1.5 mg tablet) and timing schedule for [REDACTED] are shown in Figure 1.

**Figure 1: Marketed Dosing (1.5 mg tablet) and Timing schedule for [REDACTED]**



## 2.3. Recent Cytisine Pharmacokinetic Studies

### 2.3.1. Study CYT-BIO-02-07 in Healthy Subjects

The Phase 1 CYT-BIO-02-07 study enrolled 36 healthy male subjects who received a single oral dose of 1.5 mg cytisine under fasting conditions. Blood sampling and quantitative urine collection for the determination of cytisine in plasma and urine were performed at regular intervals during the treatment period. Physical examinations were performed by the Principal Investigator or designee at the pre-study screening, after admission to the clinical center, and during the evening before drug administration to the post-study examination just prior to discharge from the clinic. Vital signs (blood pressure, heart rate, and temperature) were also performed after admission to the clinical center, during the evening before drug administration, and within 30 min before drug administration (pre-dose), and 2, 4, 12, and 24 h after drug administration during the Treatment Period.

Blood sampling for determination of cytisine concentrations was carried out in the treatment period within 30 min before (pre-dose) and at 5, 10, 20, 30, 40, and 50 min and continued at 1.0; 1.25; 1.50; 1.75; 2.0; 2.25; 2.50; 3.0; 4.0; 5.0; 6.0; 8.0, and 10.0 h after administration of 1.5 mg cytisine (one [REDACTED] tablet). Urine was collected before (baseline) and quantitatively during the intervals 0-3, 3-6, 6-9, 9-12, and 12-24 h after administration of study drug. At the end of each collection interval, individual urine portions were pooled and aliquots were withdrawn from each urine pool. Creatinine concentrations were measured for each urine pool. Plasma and urine levels of cytisine were determined using validated LC-MS/MS analytical methods. Pharmacokinetic parameters of cytisine in plasma that were estimated included  $AUC_{last}$ ,  $AUC_{inf}$ ,  $C_{max}$ ,  $T_{max}$ , and  $T_{1/2}$ .

Results showed that no measurable plasma levels of cytisine were observed 5 min after drug administration. After 10 min, a level exceeding the limit of quantitation (LOQ) was noted in 5 of 36 (14%) subjects; after 20 min, a level exceeding the LOQ was noted in 29 of 36 (81%)



subjects. At 10 h after drug administration, the cytisine plasma levels exceeded LOQ in all subjects. Mean values and other calculated PK parameters are presented in [Table 1](#) (extracted from the Study CYT-BIO-02-07 report).

**Table 1: Means of Pharmacokinetics Parameters for Cytisine**

	<b>C<sub>max</sub></b> ng/mL	<b>C<sub>last</sub></b> ng/mL	<b>AUC<sub>inf</sub></b> ng.h/mL	<b>AUC<sub>last</sub></b> ng.h/mL	<b>AUC<sub>res</sub></b> ng.h/mL	<b>AUC %</b> Ex-inf %	<b>T<sub>½</sub></b> h	<b>K<sub>a</sub></b> l/h	<b>λ<sub>z</sub></b> 1/h	<b>MRT</b> h	<b>T<sub>max</sub></b> h
Mean	15.55	2.24	73.63	59.80	13.83	18.33	4.09	2.81	0.1758	6.10	0.92
SD	6.55	0.81	21.27	16.77	7.36	6.46	0.82	1.30	0.0336	1.23	0.40
SEM	1.09	0.13	3.55	2.79	1.23	1.08	0.14	0.38	0.0056	0.21	0.07
Median	14.97	1.92	70.72	58.58	11.66	17.89	4.00	0.95	0.1735	6.02	0.83
Min	5.37	0.88	38.37	32.49	4.45	7.20	2.74	3.01	0.1090	4.30	0.33
Max	36.67	4.40	121.92	103.47	40.33	35.38	6.36	4.42	0.2531	9.65	1.75
CV%	42	36	29	28	53	35	20	46	19	20	44
Geo Mean	14.39	2.10	70.67	57.53	12.19	15.25	4.02	2.49	0.1726	5.99	0.84
CI 95%											
Lower	13.33	1.97	66.43	54.13		16.15	3.81	1.98	0.1644	5.68	0.78
Upper	17.76	2.52	80.83	65.48		20.52	4.37	3.64	0.1872	6.52	1.06
KS p value	0.65	0.22	0.64	0.90		0.61	0.89	0.282	0.9837	0.55	0.12

Mean C<sub>max</sub> (maximal plasma concentration) for the 36 subjects was 15.55 ± 6.55 ng/mL with a mean T<sub>max</sub> (time to maximal plasma concentration) of 0.92 h ± 0.40, ranging from 0.33-1.75 h. The mean AUC<sub>last</sub> (area under the concentration-time curves over 0-t period) was 59.80 h.ng/mL ± 16.77 and the AUC<sub>inf</sub> (area under the concentration-time curve from 0 to infinity) was 73.63 h.ng/mL ± 21.27. The mean AUC<sub>res</sub> was less than 20% of AUC<sub>inf</sub>. The mean T<sub>½</sub> (plasma elimination half-life) was 4.09 h ± 0.82 with a range from 2.74-6.36 h and MRT (mean residence time) was 6.10 ± 1.23 h.

Mean urine concentration of cytisine in urine samples are presented in [Table 2](#) (extracted from the Study CYT-BIO-02-07 report).

**Table 2: Mean Cytisine Urine Concentrations and Pharmacokinetic Parameters.**

	Cytisine urinary concentrations (µg)					Ae <sup>a</sup>	% recovery
	0 – 3 h	3 – 6 h	6 – 9 h	9 – 12 h	12 – 24 h		
Mean	329.02	250.58	154.25	105.28	119.62	958.75	63.92
SD	102.42	98.05	73.07	43.99	56.34	238.67	15.91
SEM	17.07	16.34	12.18	7.33	9.39	39.78	2.65
Median	336.98	235.98	134.31	94.76	119.93	896.22	59.75
Min	70.72	14.73	11.14	20.95	24.80	24.89	34.71
Max	519.35	469.20	357.90	227.97	250.63	929.79	100.24
CV%	31.13	39.13	47.37	41.78	47.10	24.89	24.89
Geo Mean	308.24	221.92	135.49	96.07	105.64	929.79	61.99
CI 95%							
Lower	294.36	217.41	129.53	90.40	100.56	877.99	58.53
Upper	363.67	283.76	178.98	120.16	138.68	1039.50	69.30

<sup>a</sup>Ae: Amount excreted

Mean cumulated values of cytisine excreted during a 24 h period evaluated as percent of administered dose was  $958.75 \pm 238.67$  µg. The results indicated that, during a 24 h period, around 64% of the oral dose was excreted in the urine as parent compound.

In summary, there were marked inter-individual differences observed in plasma concentrations as well as in all PK parameters after cytisine administration (e.g., CV exceeding 30% was noted for  $T_{max}$ ,  $C_{max}$ , and AUC). The mean half-life for cytisine was  $4.09 \text{ h} \pm 0.82$ . Cumulative amounts of cytisine were excreted in the urine within 24 h indicating that cytisine is most likely readily absorbed from the gastrointestinal tract after oral administration and may not undergo metabolic processes, since approximately 64% of the administered dose was excreted as unchanged cytisine within 24 h. More details are in the Investigator’s Brochure.

### 2.3.2. PK Study in Healthy Smoking Subjects published by the University of Auckland

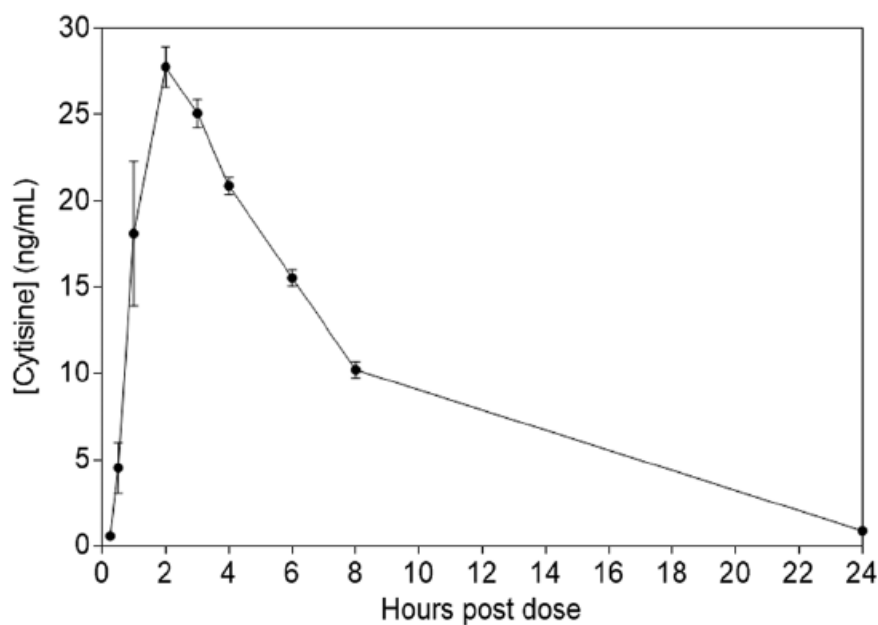
A pilot PK study was conducted at the University of Auckland in New Zealand and published by Soo Hee Jeong<sup>10</sup> et al in 2014. The study developed an analytical LC-MS assay for the detection of cytisine in plasma and urine following a single 3 mg oral dose. The LC-MS method in this study complied with limits set by US FDA guidelines including accuracy, precision, specificity, and linearity. The PK characteristics of cytisine were evaluated in seven healthy smokers after a single 3 mg oral cytisine dose (i.e. double the dose used in Study CYT-BIO-02-07) over a 24 h period. Subjects enrolled smoked an average of 10.6 cigarettes per day and had been smoking for an average of 9.5 years with only two subjects having previously made an attempt to quit smoking.

Ten blood samples were collected in heparinised tubes at the following times from each subject: 0 (just before dosing), 0.25, 0.5, 1, 2, 3, 4, 6, 8, and 24 h after cytisine administration. Blood pressure, heart rate and respiratory rate were also measured at 0 (just before dosing), 2, 4, 8, and

24 h after drug administration. After providing a blood sample at 8 h, subjects went home and returned the next morning, 24 h post-dosing. Subjects also provided spot urine samples. Non-linear mixed effects modelling (NONMEM) was used for modelling and estimating population pharmacokinetic parameters (CL, Vd). A single compartment model was fit to the data.

After a single 3 mg oral dose, cytisine was absorbed into the bloodstream, with cytisine detectable in plasma as early as 15 min after dosing (Figure 2). 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 have been achieved between 1 and 2 h for all subjects. This is broadly consistent with the estimate of 0.92 h in the Study CYT-BIO-02-07. Since the blood sampling in this study had a less frequent sampling schedule of 0.25, 0.5, 1, and 2 h, this study was less able to accurately estimate a  $T_{max}$  of approximately an hour. The mean  $C_{max}$  was 27.76 ng/mL. Following the peak plasma concentration, cytisine concentrations declined in a monophasic manner and the  $T_{1/2}$  of cytisine was calculated to be 4.8 h.

**Figure 2: Mean plasma concentrations (ng/mL) of cytisine over 24 hours**



Mean plasma concentrations (ng/mL) of cytisine over 24 hours following a single 3 mg oral dose. Values are shown as mean  $\pm$  SEM (n=7).

The values for Vd and CL were estimated to be 115 L and 16.7 L/h, respectively with standard error values 0.003.

Cytisine was still detectable in the urine collected at 24 h for all subjects (mean 24 h concentration was 428.15 ng/mL). Cytisine was detected in urine as unchanged cytisine and no metabolites were detected in plasma or in urine in any of the subjects in the study.

In summary, the logarithmic plot of mean cytisine concentration versus time after  $C_{max}$  showed a single elimination phase (i.e., no distinction was made between the distribution and elimination phase of the drug). This indicated that single dose PK of cytisine may be described using a single-compartment model. Since the apparent volume of distribution for cytisine estimated for the population in the study (115 L) was more than three times that of the blood compartment (35 L), this suggested that cytisine may distribute to other compartments very rapidly. Plasma cytisine concentrations declined with an average elimination half-life of 4.8 h. Thus, in this pilot study, the half-life for 3 mg cytisine in smokers (4.8 h) was the same for 1.5 mg cytisine in healthy non-smokers ( $4.09 \text{ h} \pm 0.82$ ). This study is described in more detail in the Investigator's Brochure.

## 2.4. Safety Summary

Given that cytisine [REDACTED] has been marketed in Central and Eastern Europe, including four Member States of the European Union (EU), for several decades, there is considerable clinical experience with this product. Over 20 million subjects have been treated, and over 10,000 subjects have taken part in clinical trials, the majority of which were conducted several years ago. [REDACTED], the manufacturer of [REDACTED], has been filing Periodic Safety Update Reports (PSURs) since 2005. The cumulative denominator in PSURs is now over 15 million exposures.

Since cytisine has a structural similarity to nicotine and is a partial agonist of nicotine acetylcholine receptors, the possible pharmacodynamic undesirable effects are manifested mainly as nicotine effects. In 6 placebo-controlled clinical trials (N=2844) 1389 subjects received cytisine. The most commonly reported adverse events in the cytisine group were effects on the gastro-intestinal system: abdominal pain upper, nausea, dyspepsia, dry mouth, vomiting, constipation and diarrhea. Nervous system and psychiatric disorders were also common, most frequently headache and dizziness, as well as somnolence and insomnia. However, the statistical analysis did not reveal any significant difference in the nervous system adverse events between the cytisine and placebo groups ( $p=0.12$ ).

Please reference the cytisine Summary of Product Characteristics (SmPC) for additional human safety information.

## 2.5. Recent Nonclinical Toxicology Studies: Estimation for Higher Human Equivalent Doses

A number of non-clinical studies with cytisine were performed more than 25 years ago and, thus, pre-dated current GLP standards. The US National Center for Complementary and Integrative Health (NCCIH) has sponsored new non-clinical GLP studies that have included 28-day repeat dosing for toxicology assessments in rats and dogs to current GLP guidance.

Daily oral dose administration of cytisine in Sprague Dawley rats at 5 or 20 mg/kg/day for 28 consecutive days resulted in clinical signs, such as drooling, in a dose dependent manner. Other clinical signs were only seen in the 20 mg/kg group including ruffled fur, hypoactivity, shoveling behaviors, and ataxia. Decreases in food consumption and body weight gain were observed in animals treated with 20 mg/kg of cytisine. No cytisine-related macroscopic and microscopic findings were observed. The no observed adverse effect level (NOAEL) of cytisine is considered to be approximately 5 mg/kg/day when given orally for 28 day consecutive days in rats. This

would calculate to an estimated Human Equivalent Dose (HED) of 0.3 mg/kg (i.e. 21 mg for a 70 kg human) when cytosine is administered for 28 consecutive days.

Daily oral dose administration of cytosine via capsules in Beagle dogs at 0.5, 1.0, or 2 mg/kg/day for 28 consecutive days resulted in adverse effects. The maximum tolerated dose (MTD) was considered to be greater than 1 mg/kg/day but less than 2 mg/kg/day. Although emesis, drooling and diarrhea were observed in some animals treated with 0.5 mg/kg cytosine, the severities were mostly slight and not dose-limiting clinically; therefore, the NOAEL was slightly less or at approximately 0.5 mg/kg/day following 28 days oral administration. This gives an estimated HED of 0.28 mg/kg (i.e. 20 mg for a 70 kg human) when cytosine is administered for 28 consecutive days.

Based on the findings in both the 28-day rat and 28-day dog toxicology studies, the established commercial dose of 1.5 mg X 6 doses/day (maximum total exposure of 9 mg/day for the first 3 days of treatment) is approximately half that of the estimated HED of ~20 mg/day for humans. The planned higher 3 mg/dose for this study yields a maximum daily dose exposure of 18 mg for the first 3 days of treatment. This again is below the calculated HED based on all of the studies as shown in [Table 3](#).

**Table 3: Calculation of Margin of Safety based on Rat and Dog Toxicology Studies**

NOAEL	C <sub>max</sub> (ng/mL)	AUC <sub>last</sub> (ng*h/mL)	HED <sup>a</sup> (mg/kg/day)	HED <sup>a</sup> (mg/day)	Human commercial Dose <sup>b</sup>	Margin of Safety based on BSA
7-day rat study: 5 mg/kg	383.5	2926	0.8	56	9 mg/day	~6X
7-day dog study: 2 mg/kg	317.5	708.5	1.1	77	9 mg/day	~8X
28-day rat study: 2 mg/kg	292.6	1071.7	0.3	21	9 mg/day	~2X
28-day dog study: 0.5 mg/kg	TBD	TBD	0.28	20	9 mg/day	~2X

<sup>a</sup> HED = Human Equivalent Dose

<sup>b</sup> Human commercial dose is 9 mg/day for the first 3 days in the 25-day schedule per commercial use.

Notes: C<sub>max</sub> and AUC are determined from the NOAEL data.

HED (mg/kg/day) = rat dose divided by 6.2 and dog dose divided by 1.8.

HED (mg/day) = HED dose (mg/kg/day) x 70 kg.

In conclusion, these margins of safety, based on HED calculations in newly conducted GLP toxicology studies support evaluating the pharmacokinetic parameters and pharmacodynamic effects of a higher 3 mg dose (maximum of 18 mg for the first 3 days) compared to the commercial dose of 1.5 mg (maximum of 9 mg for the first 3 days). In addition, these data are relevant for a future Phase 3 trial design.

### **3. RATIONALE FOR THE STUDY**

Although cytisine [REDACTED] has been marketed in Central and Eastern Europe using a standardised 25-day treatment schedule at the manufactured 1.5 mg dose level, there have been no recent pharmacokinetic evaluations of repeat dosing at 1.5 mg or 3.0 mg in adult smokers, including the elderly (>65 years of age). Therefore, this study will evaluate the detailed pharmacokinetics of 1.5 mg or 3.0 mg cytisine in adult smokers aged 18-65+ years as administered per the current [REDACTED] labeling (25-day commercial schedule). In addition, assessment for any potential effect of cytisine on QT/QTc interval prolongation will be explored and analysed in relationship to the cytisine pharmacokinetic profile as well as general safety assessments for the 3 mg dose.

### **4. STUDY OBJECTIVES**

#### **4.1. Primary Objectives**

1. To evaluate the pharmacokinetic (PK) parameters during repeat dosing of 1.5 mg or 3.0 mg cytisine when administered as the commercial 25-day schedule.
2. To evaluate the pharmacodynamic (PD) effects (e.g. reduction in smoking) with repeat dosing of 1.5 mg or 3.0 mg cytisine when administered as the commercial 25-day schedule.

#### **4.2. Secondary Objectives**

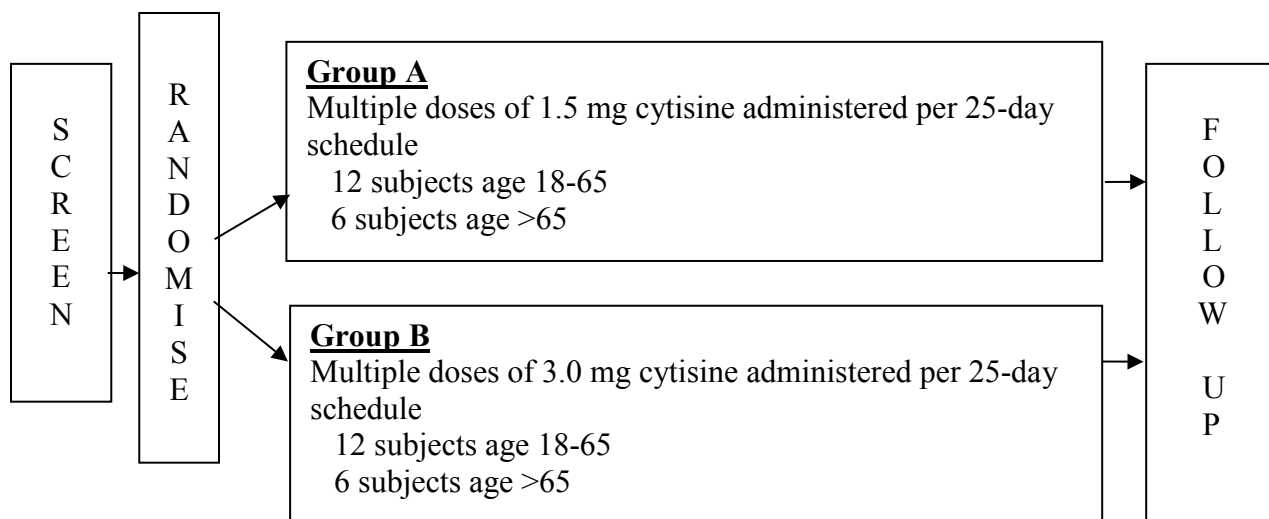
1. To compare the PK parameters and tolerability for repeat dosing of 1.5 mg and 3.0 mg cytisine during the 25-day schedule in healthy smokers at 18-65 and >65 (elderly) years of age.
2. To assess the renal elimination of cytisine via measurement of urinary concentrations of cytisine during treatment on Day 1 and Day 25.
3. To evaluate for effects on QT/QTc interval prolongation and cardiac safety during treatment on Day 1 and Day 25 in healthy smokers at 18-65 and >65 (elderly) years of age.

### **5. INVESTIGATIONAL PLAN**

#### **5.1. Study Design**

This will be an open-label, randomised, multiple-dose PK and PD study conducted in adult smokers, 18-65+ years of age as shown in [Figure 3](#):

**Figure 3: Study Design Overview**



The study will be comprised of a pre-study screen, followed by 25 days of treatment and a post-study follow-up.

**Screening (Day -28 to Day -1):** Screening assessments will be carried out within 28 days before first administration of cytisine (Section 11.2.1). Eligible subjects will then be asked to return on the evening prior to Day 1 (i.e. Day -1) for repeat of key entry testing, baseline questionnaires and provide overview of the risks of smoking and quit advice according to World Health Organization (WHO) guidelines<sup>11</sup> (Section 11.2.2). Randomisation to treatment group will occur on the morning of Day 1 followed by initiation of study treatment.

**Treatment Period (Day 1 to Day 25):** Eligible subjects will take cytisine as outlined in the [redacted] product insert (SmPC). **NOTE:** Group A subjects take 1 tablet at each timepoint (1.5 mg), Group B subjects take 2 tablets at each timepoint (3.0 mg).

**Figure 4: Dose Timing and Total Daily Dose**

Days	Regimen	Total Daily Dose		Approximate Interval
		1.5 mg Dose	3.0 mg Dose	
1-3	6 times daily	9.0 mg	18.0 mg	2 hours
4-12	5 times daily	7.5 mg	15.0 mg	2.5 hours
13-16	4 times daily	6.0 mg	12.0 mg	3 hours
17-20	3 times daily	4.5 mg	9.0 mg	4-5 hours
21-24	2 times daily	3.0 mg	6.0 mg	6 hours
25	Once daily	1.5 mg	3.0 mg	-

A total of 9 overnight stays in the clinic will be required over the 26-day study period. Subject entry into the study may be staggered in groups in order to facilitate study management activities but all subjects passing screening are to provide clinic visits as follows:

- Day -1 and Days 1-3 (4 overnight stays in clinic): Arrive at study clinic in the afternoon of Day -1, repeat select screening testing and verification of inclusion/exclusion criteria. Provide risks of smoking and quit advice according to WHO guidelines that evening.<sup>11</sup> All subjects will fast for minimum of 10 hours prior to first dose on Day 1. Randomisation to treatment Group assignment will occur on the morning of Day 1, just prior to starting treatment. On the morning of Day 1, obtain baseline blood and urine and CO level, initiate Holter monitoring, receive study drug packets, begin treatment and provide required blood and urine samples. Subjects may have breakfast after first dose and continue with a standard meal schedule. On Days 1-3, various safety assessments and blood sample collections will occur. On the morning of Day 4 complete vital signs, ECG, blood for haematology and biochemistry testing, urine for cotinine test, expired CO, completion of tobacco craving questionnaire, safety assessment and collection of the pre-dose PK blood draw. Subject will then take first Day 4 dose followed by risks of smoking and quit advice, review of diary requirements, reminder of next clinic visit date and will be discharged.
- Day 4-11: Subjects to maintain dosing as per schedule.
- Day 12-13 (1 overnight stay in clinic): Subjects return to clinic prior to last evening dose on Day 12 for pre-dose PK blood collection. Blood collections occur post final dose on Day 12 for PK. On the morning of Day 13 complete vital signs, ECG, blood for haematology and biochemistry testing, urine for cotinine test, expired CO, completion of tobacco craving questionnaire, safety assessment, and collection of the pre-dose PK blood draw. Subject will then take first Day 13 dose followed by risks of smoking and quit advice, review of diary requirements, reminder of next clinic visit date and will be discharged.
- Day 13-15: Subjects maintain dosing as per schedule.
- Day 16-17 (1 overnight stay in clinic): Subjects return to clinic prior to last evening dose on Day 16 for pre-dose PK blood collection. Blood collections occur post final dose on Day 16 for PK. On the morning of Day 17 complete vital signs, ECG, blood for haematology and biochemistry testing, urine for cotinine test, expired CO, completion of tobacco craving questionnaire, safety assessment, and collection of the pre-dose PK blood draw. Subject will then take first Day 17 dose followed by risks of smoking and quit advice, review of diary requirements, reminder of next clinic visit date and will be discharged.
- Day 17-19: Subjects maintain dosing as per schedule.
- Day 20-21 (1 overnight stay in clinic): Subjects return to clinic prior to last evening dose on Day 20 for pre-dose PK blood collection. Blood collections occur post final dose on Day 20 for PK. On the morning of Day 21 complete vital signs, ECG, blood for haematology and biochemistry testing, urine for urinalysis and cotinine test,



expired CO, completion of tobacco craving questionnaire, safety assessment, and collection of the pre-dose PK blood draw. Subject will then take first Day 21 dose followed by risks of smoking and quit advice, review of diary requirements, reminder of next clinic visit date and will be discharged.

- Day 21-23: Subjects maintain dosing as per schedule.
- Day 24-26 (2 overnight stays in clinic): Subjects return to clinic prior to last evening dose on Day 24 for pre-dose PK blood collection. Blood collections occur post final dose on Day 24 for PK. All subjects will have breakfast 30 minutes prior to final dose on Day 25. Extensive PK blood sampling, urine collections and Holter monitoring occur pre and post final dose on Day 25, with final draw 24 hours post dose (Day 26). Final discharge from clinic on Day 26 will occur after 24 hour blood draw, vital signs, ECG, haematology and biochemistry testing, serum pregnancy test for female subjects, urine for urinalysis and cotinine test, expired CO, completion of tobacco craving questionnaire, safety assessment, risks of smoking and quit advice, review of diary requirements, and reminder of post study followup visit date.
- **Post Study:** A post study follow-up visit will be conducted 6 to 8 days after last dose of cytisine. If any adverse events are recorded at post study follow up assessment, arrangements will be made with the subject, so that they are followed-up appropriately and the final outcome determined. Smoking history will be reviewed and final review of risks of smoking and quit advice provided.

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 9 weeks (screening to last subject's last visit).

## **6. SELECTION OF STUDY POPULATION**

A total of 36 subjects will be required to complete the study. Of the 36 subjects, 24 are to be 18-65 years and 12 are to be >65 years of age. An attempt will be made for 50% male and 50% female enrollment.

The study is to be conducted in adult smokers and therefore participants may receive therapeutic benefit from taking part. Outside of being active smokers, a healthy subject population with carefully considered inclusion/exclusion criteria will ensure 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. Regular moderate cigarette smokers (minimum 10 cigarettes per day) who want to stop smoking.

2. Urine cotinine >500 ng/mL.
3. Expired air carbon monoxide (CO) > 11 parts per million (no cigarette 1 hour before test).
4. Healthy males and females 18-65+ 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 cytisine.
  - 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 cytisine.
5. Subject with no clinically significant abnormal serum biochemistry, haematology and urine examination values within 28 days before the first dose of cytisine.
6. Subject with negative urinary drugs of abuse screen, determined within 28 days before the first dose of cytisine (a positive alcohol result may be repeated at Investigator's discretion).
7. Subject with negative human immunodeficiency virus (HIV), hepatitis B surface antigen (Hep B) and hepatitis C virus antibody (Hep C) results.
8. 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 cytisine.
9. Subject with no clinically significant abnormalities in vital signs (systolic blood pressure between 90-150 mmHg (age 18-65) and 90-160 mmHg (age >65), 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 cytisine.
10. Subject must be available to complete the study (including in-clinic stays and post study follow-up) and comply with study restrictions.
11. Subject must provide written informed consent to participate in the study.

### **6.1.2. To be Re-Confirmed Prior to Start of Dosing (Day -1)**

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).
3. Female subject has a negative pregnancy test.

## **6.2. Exclusion Criteria**

### **6.2.1. To be Confirmed at Screening**

1. Treatment with smoking cessation medications (bupropion, varenicline, any nicotine replacement therapy) within 8 weeks of first dose of cytisine.
2. Use of other forms of nicotine (e-cigarettes, smokeless tobacco) within 8 weeks of first dose of cytisine or are planning to use these products during study.
3. Known hypersensitivity/allergy reaction to varenicline, other cytisine-derivatives or any of the excipients in the [REDACTED] formulation.
4. History of severe hypersensitivity reactions to any other drugs.
5. Current treatment with antihypertensive medicinal products, statins, tuberculostatics, cholinomimetics or anticholinesterase medicinal products.
6. 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).
7. Female subjects who are breast feeding.
8. Difficulty in donating blood on either arm or known history.
9. History of alcoholism or drug abuse within last 2 years.
10. Use of 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 cytisine, unless in the opinion of the Principal Investigator the medication will not interfere with the study procedures or compromise subject safety.
11. Participated in any investigational drug clinical trial within the previous 3 months or a marketed drug trial within the previous 30 days prior to randomisation on Day 1.
12. 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 cytisine.
13. Inability to communicate well with Principal Investigator or designees (i.e., language problem, poor mental development or impaired cerebral function).
14. Any other condition that the Principal Investigator considers making the subject unsuitable for this study.

### **6.2.2. To be Re-Confirmed Prior to Start of Dosing (Day -1):**

1. Development of any exclusion criteria since screening.

2. Use of non-prescription drugs, including vitamins, herbal and dietary supplements since screening, unless in the opinion of the Principal Investigator or designee the medication will not interfere with the study procedures or compromise subject safety.
3. Participation in a clinical study since the screening visit.
4. Donation of 450 mL or more blood since the screening visit.

### **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.

The Principal 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 36 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 Principal Investigator (or designee) and Sponsor.

## **7. RESTRICTIONS 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 be required to provide a total of 9 overnight stays in the clinic over a 26-day period.

## **7.2. Smoking, Diet and Fluid Restrictions**

### **7.2.1. Smoking**

There will be minimal restrictions to smoking during days of confinement, such as all smoking must occur within designated area(s) only and subjects cannot smoke during active PK sampling period (i.e. subjects must have their last cigarette a minimum of 2 hours before dosing and can only start smoking after final PK sample). Subjects will go for a cigarette as a group and will need to have their last cigarette before 23:00 while they stay in the clinic. Clinic staff will review these guidelines with subjects during confinement and will monitor compliance.

### **7.2.2. Meal Times**

During days of confinement, subjects will be provided standard meals (breakfast, lunch, dinner, snacks) at specific times, concurrent with study treatment dosing when possible. Subjects to be fasting prior to the first Day 1 dose and will be fed breakfast 30 minutes prior to the last Day 25 dose.

### **7.2.3. Fluid Intake**

Prior to PK sampling only, 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.

### **7.2.4. Alcohol Intake**

The consumption of alcohol will be limited to a maximum of 2 units (50 mL spirits, 500 mL beer or lager or 250 mL wine) per day from 7 days prior to the first dose of cytisine. Alcohol will be avoided completely for at least 2 days prior to the first dose of cytisine on Day 1 and again on Day 25. Alcohol will be avoided completely for 24 hours prior to each day of confinement for PK sampling.

### **7.2.5. Caffeine**

Food or drink containing caffeine, including coffee, tea, cola, energy drinks or chocolates will be avoided completely for 24 hours prior to each day of confinement for PK sampling.

### **7.2.6. Poppy and Sesame Seeds**

Subjects must not eat food containing poppy or sesame seeds for 3 days before drug of abuse testing, as consumption can lead to a positive opiate result in the drugs of abuse test.

### **7.2.7. Grapefruit Juice and Other Restrictions**

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 avoided completely for 24 hours prior to each day of confinement for PK sampling.

Each subject will be questioned on the specific points at admission to the clinical unit as appropriate. If a subject admits a non-compliance with these restrictions, the Principal Investigator or designee 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. Blood Donation**

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

#### **7.3.2. Contraception**

To prevent pregnancy female subjects of childbearing potential and male subjects with female partners of child bearing potential must take / agree to 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 cytisine.

#### **7.3.3. Sperm Donation**

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

## 8. INVESTIGATIONAL MEDICINAL 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 4).

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

Cytisine should be administered orally according to the dose timing schedule (see [Figure 4](#)) with each dose to be either [REDACTED] taken with [REDACTED] ([REDACTED]) and, when possible, with a meal or snack.

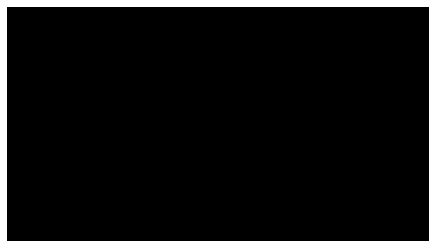
<b>Name of the Product:</b>	Cytisine 1.5 mg, film coated tablets [REDACTED]
<b>Strengths:</b>	[REDACTED]
<b>Dosage form:</b>	[REDACTED]
<b>Route/mode of administration:</b>	Oral
<b>Dosing Schedule:</b>	(See <a href="#">Figure 4</a> )
<b>Dosing instructions:</b>	Depending of Treatment Group assignment, [REDACTED]
<b>Manufacturer/Marketing Authorisation Holder:</b>	[REDACTED] [REDACTED] [REDACTED]

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

### 8.2. Receipt and Storage

Cytisine will be supplied by the Sponsor as commercial product in a format suitable for study specific relabeling.

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



Cytisine will be stored under quarantine in a segregated, study-specific area, at or below 25 °C 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**

Cytisine will be supplied to study subjects in its commercial configuration, as manufactured by [REDACTED]

Cytisine 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 standard operating procedure #BD/324/13/29.

### **8.4. Administration**

Each subject will receive specified allocations of cytisine periodically during the treatment period that will allow for correct dosing as assigned at randomisation (1.5 mg or 3.0 mg dose level). Cytisine allocations will be documented and actual dosing (and time of dosing) will be documented by each subject in their diary.

### **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 Group A or Group B**

Subjects will be randomised to treatment dosing (1.5 mg or 3.0 mg) according to a code produced by [REDACTED] using the PROC PLAN procedure of SAS<sup>®</sup> version 9.1.3 or higher. Subjects will be numbered sequentially from 001 (i.e. 001, 002 etc.). Subjects will be stratified for age (elderly > 65 years: yes vs no) to assure that each treatment dosing has 6 elderly subjects and 12 non-elderly subjects. 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 either 1.5 mg or 3.0 mg cytisine.

### **8.8. Selection and Timing of Dose for Each Subject**

First dose will be administered at approximately 9.00 a.m. on Day 1. Additional doses will be as per the Dosing Schedule (see [Figure 4](#)). A target time will be provided for each dose in the diary and actual time of dose will be recorded. For Days where dosing is every 2 to 3 hours, dosing should be  $\pm$  10 min within the targeted time. For Days where dosing is every 4-6 hours, dosing should be  $\pm$  30 min within the targeted time.



## **8.9. Dose Modifications for 3.0 mg Dose or Elderly Subjects (>65)**

Dose modifications should not be necessary, unless a severe adverse event warrants holding or missing a dose as outlined below during the first 1 – 16 days in the 3 mg cytisine dosage group or at any time in elderly subjects.

Cytisine at higher levels in toxicology studies elicited adverse reactions primarily on the gastrointestinal system such as vomiting or diarrhea. Based on the HED calculations in newly conducted GLP toxicology studies (see Section 2.5), the safety margin supports the 3 mg dose (maximum of 18 mg for the first 3 days). The commercial dose of 1.5 mg (maximum of 9 mg for the first 3 days) has been well tolerated with no or minimal gastrointestinal symptoms. However, if a subject has nausea that results in vomiting or has minimal diarrhea progressing to severe diarrhea, the Principal Investigator/Sponsor Medical Monitor can delay, hold or skip doses. Dosing should resume within 48 hours when the adverse event has subsided to a moderate grade (see Table 8) or minimal symptoms. Dosing should then resume on the same schedule without modification. If the adverse event has not subsided to at least a moderate grade within 48 hours, the subject should be withdrawn from further study treatment.

For all subjects randomised to the 3.0 mg dosing schedule, if a severe adverse event reoccurs during the Day 1-16 dosing, then dosing should be again held until the adverse event has subsided to a moderate grade or minimal symptoms within a 48-hour period. Dosing should then be reduced as specified in Table 5. If the severe adverse event reoccurs after reducing the dose, the subject should be withdrawn from further study treatment. If the severe adverse event does not reoccur, the patient can continue the study treatment with dosing at the 1.5 mg level as shown in Table 5.

**Table 5: Dose Modification for 3.0 mg Dose Group**

Days	Regimen	Total Daily Dose	Dose Modifications After Recurrence
1-3	6 times daily	18.0 mg	9 mg total daily dose (Reduce to one 1.5 mg cytisine tablet 6 times daily and follow the remaining schedule as for the 1.5 mg dosing group).
4-12	5 times daily	15.0 mg	7.5 mg total daily dose (Reduce to one 1.5 mg cytisine tablet 5 times daily and follow the remaining schedule as for the 1.5 mg dosing group).
13-16	4 times daily	12.0 mg	6 mg total daily dose (Reduce to one 1.5 mg cytisine tablet 4 times daily and follow the remaining schedule as for the 1.5 mg dosing group).
17-20	3 times daily	9.0 mg	No dose modification
21-24	2 times daily	6.0 mg	No dose modification
25	Once daily	3.0 mg	No dose modification

For elderly subjects in the 1.5 mg dosing schedule, if a severe adverse event reoccurs at any time, then dosing should be held until the adverse event has subsided to a moderate grade or minimal symptoms within a 48-hour period. Dosing should then be reduced by one dose per day. If the severity of the event then reoccurs, the subject should be withdrawn from further study treatment.

### 8.10. 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 all samples will be conducted without information on treatment.

## 9. PRIOR AND CONCOMITANT THERAPY

**Prior Medication:** 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 cytisine, unless in the opinion of the Principal Investigator and Sponsor's Medical Monitor the medication will not interfere with the study procedures or would compromise subject safety. Non-prescription drugs, including vitamins, herbal and dietary supplements taken during the 14 days before the first dose of cytisine, and the reason for taking them, will be noted in the subject's CRF.

Inclusion of subjects who are on prescription medications or 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:** New prescription or other non-prescription drugs, including smoking cessation medications, tuberculostatics, cholinomimetics or anticholinesterase medicinal products, statins or antihypertensive medicinal products, 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 cytisine taken while in the clinic will be taken under supervision and a hand and mouth check conducted. Subjects will be required to maintain a diary to document dosing times and provide medication packets for tablet accountability at each clinic visit. These will be reviewed by clinic staff during clinic visits.

## 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 randomised according to the randomisation schedule on Day 1. Subjects will receive their initial allocation of study medication on Day 1. The subjects will be required to make a total of 9 overnight clinic stays over a 26-day period. All subjects are required to complete a follow-up visit 6-8 days post last dose.

### 11.1. Procedure Schedule

Table 6 provides a summary of required study evaluations. Screening evaluations are to occur within a 28-day interval from initiation of screening evaluations to Day -1, just prior to randomisation on Day 1.

**Table 6: Schedule of Study Procedures**

Study Day	Screening	Study Day										Post-Study
	-28 to -2	-1 <sup>1</sup>	1-4 <sup>1</sup>	4-11	12-13 <sup>2</sup>	13-15	16-17 <sup>2</sup>	17-19	20-21 <sup>2</sup>	21-23	24-26 <sup>3</sup>	6-8 days after last dose
Written informed consent	X											
Number of cigarettes smoked in previous 24 hours	X	X										
Daily Smoking and Study Medication Diary <sup>4</sup>		X	X	X	X	X	X	X	X	X	X	X
Demographic data	X											
Randomisation			X <sup>5</sup>									
Vital signs	X	X <sup>6</sup>	X <sup>6</sup>		X <sup>6</sup>		X <sup>6</sup>		X <sup>6</sup>		X <sup>6</sup>	
Medical history	X	X <sup>7</sup>										
Prior and concomitant medication	X	X	X		X		X		X		X	X
Physical examination	X											
12-lead ECG	X	X	X <sup>8</sup>		X <sup>8</sup>		X <sup>8</sup>		X <sup>8</sup>		X <sup>8</sup>	
Holter monitor for continuous ECG readings <sup>9</sup>			X								X	
Pregnancy test for all females	X Serum	X Urine									X Serum	
FSH (females of non-childbearing potential only)	X											
Haematology <sup>10</sup>	X		X		X		X		X		X	
Biochemistry <sup>10</sup>	X		X		X		X		X		X	
Virology (HIV, Hep-B, Hep-C)	X											
Urinalysis	X										X	
Drugs-of-abuse tests in urine	X	X										
Fagerström nicotine dependence		X									X	
Urine cotinine <sup>11</sup>	X		X		X		X		X		X	
Expired air CO <sup>11</sup>	X		X		X		X		X		X	

Verification of eligibility criteria	X	X	X									
Tobacco craving questionnaire		X	X		X		X		X		X	
Risks of smoking and quit advice <sup>12</sup>		X	X		X		X		X		X	X
IMP (cytisine) administration <sup>13</sup>			X	X	X	X	X	X	X	X	X	
Plasma collection for PK analysis <sup>14</sup>			X		X		X		X		X	
Urine collection for PK analysis <sup>15</sup>			X								X	
Adverse events monitoring <sup>16</sup>			X		X		X		X		X	X

<sup>1</sup> 4 overnight stays in clinic, starting evening of Day -1.

<sup>2</sup> 1 overnight stay in clinic.

<sup>3</sup> 2 overnight stays in clinic.

<sup>4</sup> Subject to record number of cigarettes smoked in past 24 hours on a daily basis throughout study, actual time of each medication dose and record any adverse events. Information to be reviewed and documented during clinic days. Subject to also maintain record of number of cigarettes smoked in each 24 hour period from Day 26 until final Post-Study Visit (see Section 13.9).

<sup>5</sup> Pre-First Dose on Day 1

<sup>6</sup> Vital signs (supine blood pressure, pulse rate and oral temperature) will be recorded at pre-dose and again prior to discharge from clinic. Vital signs will also be recorded 3 hours post last dose on Days 1, 2, 3, 12, 16, 20 and 24.

<sup>7</sup> Clinically relevant changes will be reported as adverse events.

<sup>8</sup> Obtain 12-lead ECG (triplicate) at any time if clinically indicated during clinic visits. Repeat 12-lead ECG and assess prior to discharge.

<sup>9</sup> Attach ECG Holter monitor and begin recording prior to first dose on Day 1 and continue for 24 hours (discontinue just prior to first dose on Day 2. Repeat again by starting Holter monitor prior to dose on Day 25 and record for 24 hours.

<sup>10</sup> Haematology and biochemistry testing to be completed prior to discharge on Days 4, 13, 17, 21, 26

<sup>11</sup> Urine cotinine and expired CO testing to be obtained at screen and prior to discharge on Days 4, 13, 17, 21, 26.

<sup>12</sup> Each subject given advice about the risks of smoking and quitting. This will take the form of a brief interview following WHO evidence-based recommendations on the treatment of tobacco dependence on Day -1 and reminders prior to discharge on Days 4, 13, 17, 21, 26 and on the final post-study visit.

<sup>13</sup> IMP administration to follow 25-day dosing schedule (see Figure 4).

<sup>14</sup> Plasma collection for PK analysis is outlined in Section 12.1.

<sup>15</sup> Urine to be collected for PK on Day 1 and Day 25 as follows: pre-dose and 0-24 hours.

<sup>16</sup> Adverse event recording to begin upon admissions on Day -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.

1. Written informed consent.
2. Document actual number of cigarettes smoked in the past 24 hours.
3. Expired CO.
4. Demographic data.
5. Medical history data.
6. Prior and concomitant medication.
7. Physical examination, including height and weight.
8. Vital signs (supine blood pressure, pulse rate and oral temperature).
9. 12-lead ECGs (triplicate).
10. Clinical laboratory safety tests (biochemistry, haematology, urinalysis, urine cotinine, virology, drugs of abuse, serum pregnancy for females and FSH for females of non-childbearing potential).
11. Verification of eligibility criteria.

### **11.2.2. Day -1**

All subjects will check into the research facility and upon arrival to the clinic, the following procedures will be performed:

1. Vital signs (supine blood pressure, pulse rate and oral temperature).
2. Update any medical history or concomitant medications.
3. Repeat urine testing for drugs of abuse and urine pregnancy test for female subjects.
4. Obtain 12-lead ECG (triplicate).
5. Verification of eligibility criteria.
6. Record number of cigarettes smoked in past 24 hours and instruct subject how to document cigarette smoking and dosing times in the daily smoking/medication diary (see Section 13.9).
7. Subject to complete the Fagerström nicotine dependence questionnaire (see Appendix 2).
8. Subject to complete tobacco craving questionnaire (see Appendix 3).
9. Each subject given advice about the risks of smoking and quitting. This will take the form of a brief interview following WHO evidence-based recommendations on the treatment of tobacco dependence.
10. Each subject must fast for minimum of 10 hours prior to first dose on Day 1.

### 11.2.3. Day 1 to Morning of Day 4

The results of screening for each subject must be reviewed by the Principal Investigator or designee prior to randomisation to assure eligibility criteria have been met.

#### On Day 1:

1. Vital signs (supine blood pressure, pulse rate and oral temperature).
2. Verify and document any baseline clinically relevant adverse events.
3. Randomise subject to treatment group. Provide IMP and instructions for administration, reviewing dosing schedule and documentation requirements in the study diary.
4. Attach ECG Holter monitor, checking to ensure recording has started. Note: Holter monitors to remain attached to subjects approximately 24 hours, discontinued just prior to first dose on Day 2.
5. Obtain pre-dose blood sample prior to first dose and PK sampling after first dose (Section 12.1).
6. First dose to be taken prior to breakfast (minimum fasting of 10 hours). Breakfast and standard meal schedule to begin after taking first dose.
7. Obtain urine PK samples starting prior to first dose and continuing for 24 hours (Section 12.2).
8. Subject to take 6 doses of cytisine as outlined in Figure 4.
9. Obtain pre-last dose PK blood sample and additional PK sampling after last dose (Section 12.1).
10. Record vital signs (supine blood pressure, pulse rate and oral temperature) at 3 hours after the last dose (Note: this is the estimated highest  $C_{max}$  timepoint for the daily dosing).
11. Obtain 12-lead ECG (triplicate) at any time if clinically indicated.
12. Record any adverse events and update concomitant medications.
13. The number of cigarettes smoked and actual dosing times will be recorded in the CRF.
14. All subjects will remain in the clinic overnight.

#### On Day 2:

1. Discontinue Holter monitoring approximately 24 hours after initial dose taken on Day 1 (just prior to first dose on Day 2).
2. Subject to take 6 doses of cytisine as outlined in Figure 4.
3. Obtain pre-last dose PK blood sample and additional PK sampling after last dose (Section 12.1).
4. Record vital signs (supine blood pressure, pulse rate and oral temperature) at 3 hours after the last dose (Note: this is the estimated highest  $C_{max}$  timepoint for the daily dosing).
5. Obtain 12-lead ECG (triplicate) at any time if clinically indicated.

6. Record any adverse events and update concomitant medications.
7. The number of cigarettes smoked and actual dosing times will be recorded in the CRF.
8. All subjects will remain in the clinic overnight.

**On Day 3:**

1. Subject to take 6 doses of cytisine as outlined in [Figure 4](#).
2. Obtain pre-last dose PK blood sample and additional PK sampling after last dose (Section [12.1](#)).
3. Record vital signs (supine blood pressure, pulse rate and oral temperature) at 3 hours after the last dose (Note: this is the estimated highest  $C_{max}$  timepoint for the daily dosing).
4. Obtain 12-lead ECG (triplicate) at any time if clinically indicated.
5. Record any adverse events and update concomitant medications.
6. The number of cigarettes smoked and actual dosing times will be recorded in the CRF.
7. All subjects will remain in the clinic overnight.

**On Day 4:**

1. Obtain pre-dose PK blood sample prior to Day 4 dose (Section [12.1](#)).
2. Vital signs (supine blood pressure, pulse rate and oral temperature).
3. Obtain 12-lead ECG (triplicate).
4. Obtain blood for haematology and biochemistry testing.
5. Obtain urine for cotinine test and expired air CO prior to discharge.
6. Subject to complete tobacco craving questionnaire (see [Appendix 3](#)).
7. Record any adverse events and update concomitant medications.
8. Subject to take first Day 4 dose.
9. Subject given advice about the risks of smoking and quitting.
10. Remind subject to record number of cigarettes smoked daily and actual dosing times in diary, discharge subject from clinic and schedule return for Day 12.

**11.2.4. Day 4 through Day 11**

1. Subject to maintain dosing schedule (5 times daily).
2. Subject to record number of cigarettes smoked and actual dosing times daily in diary (see Section [13.9](#)).
3. Subject to maintain adverse event log.
4. Subject to bring medication packets and diary to clinic on Day 12.



### **11.2.5. Day 12 to Morning of Day 13**

#### **On Day 12:**

1. Subject will check into the research facility in the afternoon, just prior to the last Day 12 dose.
2. Obtain pre-dose PK blood sample prior to the last Day 12 dose. Blood samples will then be collected and processed as outlined in (Section 12.1) after the last Day 12 dose of cytisine.
3. Record vital signs (supine blood pressure, pulse rate and oral temperature) at 3 hours after the last dose (Note: this is the estimated highest  $C_{max}$  timepoint for the daily dosing).
4. Obtain 12-lead ECG (triplicate) if clinically indicated.
5. Record any adverse events and update concomitant medications.
6. Review smoking/medication diary for completeness. The number of cigarettes smoked and actual dosing times will be recorded in the CRF.
7. All subjects will remain in the clinic overnight.

#### **On Day 13:**

1. Obtain pre-dose PK blood sample prior to initial dose (Section 12.1).
2. Vital signs (supine blood pressure, pulse rate and oral temperature).
3. Obtain 12-lead ECG (triplicate).
4. Obtain blood for haematology and biochemistry testing.
5. Obtain urine for cotinine test and expired air CO prior to discharge.
6. Subject to complete tobacco craving questionnaire (see Appendix 3).
7. Record any adverse events and update concomitant medications.
8. Subject to take first Day 13 dose in clinic.
9. Subject given advice about the risks of smoking and quitting.
10. Remind subject to record number of cigarettes smoked and actual dosing times daily in diary, discharge subject from clinic and schedule return for Day 16.

### **11.2.6. Day 13 through Day 15**

1. Subject to maintain dosing schedule (4 times daily).
2. Subject to record number of cigarettes smoked daily and actual dosing times in diary.
3. Subject to maintain adverse event log.
4. Subject to bring medication packets and diary to clinic on Day 16.

### **11.2.7. Day 16 through Morning of Day 17**

#### **On Day 16:**

1. Subject will check into the research facility in the afternoon, just prior to the last Day 16 dose.
2. Obtain pre-dose PK blood sample prior to the last Day 16 dose. Blood samples will then be collected and processed as outlined in (Section 12.1) after the last Day 16 dose of cytisine.
3. Record vital signs (supine blood pressure, pulse rate and oral temperature) at 3 hours after the last dose (Note: this is the estimated highest  $C_{max}$  timepoint for the daily dosing).
4. Obtain 12-lead ECG (triplicate) if clinically indicated.
5. Record any adverse events and update concomitant medications.
6. Review smoking/medication diary for completeness. The number of cigarettes smoked and actual dosing times will be recorded in the CRF.
7. All subjects will remain in the clinic overnight.

#### **On Day 17:**

1. Obtain pre-dose PK blood sample prior to initial dose (Section 12.1).
2. Vital signs (supine blood pressure, pulse rate and oral temperature).
3. Obtain 12-lead ECG (triplicate).
4. Obtain blood for haematology and biochemistry testing.
5. Obtain urine for cotinine test and expired air CO prior to discharge.
6. Subject to complete tobacco craving questionnaire (see Appendix 3).
7. Record any adverse events and update concomitant medications.
8. Subject to take first Day 17 dose in clinic.
9. Subject given advice about the risks of smoking and quitting.
10. Remind subject to record number of cigarettes smoked daily and actual dosing times in diary, discharge subject from clinic and schedule return for Day 20.

### **11.2.8. Day 17 through Day 19**

1. Subject to maintain dosing schedule (3 times daily).
2. Subject to record number of cigarettes smoked and actual dosing times daily in diary (see Section 13.9).
3. Subject to maintain adverse event log.
4. Subject to bring medication packets to clinic on Day 20.

### **11.2.9. Day 20 through Morning of Day 21**

#### **On Day 20:**

1. Subject will check into the research facility in the afternoon, just prior to the last Day 20 dose.
2. Obtain pre-dose PK blood sample prior to the last Day 20 dose. Blood samples will then be collected and processed as outlined in (Section 12.1) after the last Day 20 dose of cytisine.
3. Record vital signs (supine blood pressure, pulse rate and oral temperature) at 3 hours after the last dose (Note: this is the estimated highest  $C_{max}$  timepoint for the daily dosing).
4. Obtain 12-lead ECG (triplicate) if clinically indicated.
5. Record any adverse events and update concomitant medications.
6. Review smoking/medication diary for completeness. The number of cigarettes smoked and actual dosing times will be recorded in the CRF.
7. All subjects will remain in the clinic overnight.

#### **On Day 21:**

1. Obtain pre-dose PK blood sample prior to initial dose (Section 12.1).
2. Vital signs (supine blood pressure, pulse rate and oral temperature).
3. Obtain 12-lead ECG (triplicate).
4. Obtain blood for haematology and biochemistry testing.
5. Obtain urine for cotinine test and expired air CO prior to discharge.
6. Subject to complete tobacco craving questionnaire (see Appendix 3).
7. Record any adverse events and update concomitant medications.
8. Subject to take first Day 21 dose in clinic.
9. Subject given advice about the risks of smoking and quitting.
10. Remind subject to record number of cigarettes smoked daily and actual dosing times in diary, discharge subject from clinic and schedule return for Day 24.

### **11.2.10. Day 21 through Day 23**

1. Subject to maintain dosing schedule (2 times daily).
2. Subject to record number of cigarettes smoked and actual dosing times daily in diary.
3. Subject to maintain adverse event log.
4. Subject to bring medication packets and diary to clinic on Day 24.

### 11.2.11. Day 24 through Day 26

#### On Day 24:

1. Subject will check into the research facility in the afternoon, just prior to the last Day 24 dose.
2. Obtain pre-dose PK blood sample prior to the last Day 24 dose. Blood samples will then be collected and processed as outlined in (Section 12.1) after the last Day 24 dose of cytisine.
3. Repeat vital signs (supine blood pressure, pulse rate and oral temperature) at 3 hours after the last dose (Note: this is the estimated highest  $C_{max}$  timepoint for the daily dosing).
4. Obtain 12-lead ECG (triplicate) if clinically indicated.
5. Record any adverse events and update concomitant medications.
6. The number of cigarettes smoked and actual dosing times will be recorded in the CRF.
7. All subjects will remain in the clinic overnight.

#### On Day 25:

1. All subjects to have breakfast 30 minutes prior to final dose.
2. Attach ECG Holter monitor, checking to ensure recording has started. NOTE: Holter monitors to remain attached to subjects approximately 24 hours, discontinued on Day 26.
3. Record Vital signs (supine blood pressure, pulse rate and oral temperature).
4. Obtain pre-dose PK blood sample (Section 12.1).
5. Begin urine collection for PK as outlined in (Section 12.2).
6. Subject to take final Day 25 dose of cytisine (documenting time of dose in diary) after breakfast and begin extensive PK blood and urine sampling as outlined in (Section 12.1) throughout day and up through 24 hours post.
7. Obtain 12-lead ECG (triplicate) if clinically indicated.
8. Record any adverse events and update concomitant medications.
9. The number of cigarettes smoked will be recorded in the CRF.
10. All subjects will remain in the clinic overnight.

#### On Day 26:

1. Complete final PK blood draw and urine collection (Section 12.1).
2. Obtain blood and urine for haematology, biochemistry, serum pregnancy test (females only) and urinalysis (Section 13.4.1).
3. Vital signs (supine blood pressure, pulse rate and oral temperature).
4. 12-lead ECGs (triplicate).
5. Obtain urine for cotinine test and expired air CO prior to discharge.

6. Subject to complete tobacco craving questionnaire (see [Appendix 3](#)).
7. Record any adverse events and update concomitant medications.
8. Subject given advice about the risks of smoking and quitting.
9. Remind subject to record number of cigarettes smoked daily in diary, discharge subject from clinic and schedule follow up visit (within 6-8 days).

### 11.2.12. Post Study Follow-up

A Post-Study Follow-up visit will occur 6-8 days after last dosing to document any changes in concomitant medication(s) and adverse event(s) observed/recorded at discharge as well as any new concomitant medications or adverse events. If any new adverse events are recorded at this follow-up visit, arrangements will be made with the subject to come into the clinic for assessment and then followed until outcome determined. Subject's diary will be reviewed and collected (Note: diary will be used as source document for smoking history, actual dosing times and any self-reported adverse events). Subject given advice about the risks of smoking and quitting.

## 12. DRUG CONCENTRATION MEASUREMENTS

### 12.1. Pharmacokinetic Blood Sampling

A total of 95 venous blood samples will be collected over a 26-day period (approximate volume of 3.0 mL each, total volume of approximately 285 mL) into pre-cooled lithium heparin tubes, according to the following schedule:

**Table 7: Blood Sampling for Pharmacokinetic Assessments**

Test Period	Blood Sample Number	Time from Dosing
Day 1 (first dose)	1	Pre-Dose (within 60 minutes prior to dosing)
	2	00:30
	3	00:45
	4	01:00
	5	01:15
	6	01:30
	7	02:00
Day 1 (last dose)	8	Pre-Last Dose
	9	00:30
	10	01:00
	11	01:30

<b>Test Period</b>	<b>Blood Sample Number</b>	<b>Time from Dosing</b>
	12	02:00
	13	02:30
	14	03:00
	15	03:30
	16	04:00
	17	05:00
Day 2 (last dose)	18	Pre-Last Dose
	19	00:30
	20	01:00
	21	01:30
	22	02:00
	23	02:30
	24	03:00
	25	03:30
	26	04:00
	27	05:00
Day 3 (last dose)	28	Pre-Last Dose
	29	00:30
	30	01:00
	31	01:30
	32	02:00
	33	02:30
	34	03:00
	35	03:30
	36	04:00
	37	05:00
Day 4 (prior to first dose)	38	Pre-Dose
Day 12 (last dose)	39	Pre-Last Dose
	40	01:00
	41	01:30
	42	02:00

<b>Test Period</b>	<b>Blood Sample Number</b>	<b>Time from Dosing</b>
	43	02:30
	44	03:00
	45	03:30
	46	04:00
Day 13 (prior to first dose)	47	Pre-Dose
Day 16 (last dose)	48	Pre-Last Dose
	49	01:00
	50	01:30
	51	02:00
	52	02:30
	53	03:00
	54	03:30
	55	04:00
Day 17 (prior to first dose)	56	Pre-Dose
Day 20 (last dose)	57	Pre-Last Dose
	58	01:00
	59	01:30
	60	02:00
	61	02:30
	62	03:00
	63	03:30
	64	04:00
Day 21 (prior to first dose)	65	Pre-Dose
Day 24 (last dose)	66	Pre-Last Dose
	67	01:00
	68	01:30
	69	02:00
	70	02:30
	71	03:00
	72	03:30

<b>Test Period</b>	<b>Blood Sample Number</b>	<b>Time from Dosing</b>
	73	04:00
Day 25	74	Pre-dose
	75	00:30
	76	00:45
	77	01:00
	78	01:15
	79	01:30
	80	01:45
	81	02:00
	82	02:15
	83	02:30
	84	02:45
	85	03:00
	86	03:15
	87	03:30
	88	03:45
	89	04:00
	90	05:00
	91	06:00
	92	08:00
	93	10:00
94	12:00	
Day 26	95	24:00 (post Day 25 dose)

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, immediately placed on ice and then centrifuged within 60 minutes of draw 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 -20°C, pending analysis.

## **12.2. Pharmacokinetic Urine Collection**

Two urine samples will be collected during treatment on Day 1 and on Day 25 as follows:

- Pre-dose (pre-dose urine samples will be taken upon waking up)
- 0-24 hour

The urine collection containers will be identified with a bar coded label bearing details of the study number, subject number, study period number (Day 1 or Day 25), 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 the 24 hour collection the volume excreted will be recorded and 2 x 10 mL aliquots retained for determination of cytosine 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.

The sample analysis will only begin after the last sample of a study subject is collected. All samples from each subject will be analysed 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.

## **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 reanalysed.

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 current smokers who are healthy by inclusion and exclusion criteria are eligible for the study. 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 designee will assess the clinical significance of results of ECG readings and laboratory investigations for haematology and serum chemistry values outside the defined normal ranges, as provided by the laboratory. All changes from screening during the study and meet clinically-significant definitions for laboratory adverse events will be recorded as an adverse event.

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

On each day where subjects are in the clinic, at least one physician (Principal Investigator or designee) will physically remain at the clinical site, 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, the Principal Investigator or designee 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 visit 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 Principal 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 unauthorised investigational product or in the SmPC for an authorised 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 hospitalisation or prolongs existing inpatient’s hospitalisation
- 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 jeopardise 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 hospitalisation.

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 **hospitalisation** or prolongation of existing hospitalisation 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 the Principal Investigator or designee in accordance with the definitions below.
- Relationship to IMP to be assessed by the Principal Investigator or designee 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 the Principal Investigator or designee to describe the severity of all AEs (including clinically-significant laboratory AEs) as shown in [Table 8](#). Only 1 severity grade will be used for each AE (e.g. mild - moderate is not acceptable).

**Table 8: Adverse Event Severity**

Severity of AE	Definition
Mild	No interference with activity
Moderate	Some interference with activity requiring no or minimal medical intervention
Severe	Prevents or significantly interferes with daily activity and requires medical intervention

If an adverse event has multiple aspects, the aspect in the highest intensity will be graded. It is emphasised 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 Principal Investigator or designee, according to best medical judgment as shown in [Table 9](#).

**Table 9: Assessment of Causal Relationship to Cytisine**

<b>Catagory</b>	<b>Description</b>
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 Principal Investigator or designee 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]

[REDACTED]

██████████ will notify the Sponsor and Sponsor's Medical Monitor 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:

- Principal 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, Healthcare products Regulatory Agency (MHRA) and Sponsor in accordance with applicable regulatory requirements for expedited reporting. It is ██████████ 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 Principal Investigator or designee 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 prior to discharge at each clinic visit 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 the Principal Investigator or designee 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<sup>®</sup> collection tube (or equivalent) and analysed using the Siemens Advia 2120<sup>®</sup> or Siemens Advia 120<sup>®</sup>.

**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<sub>3</sub>), creatinine (CREA) and urea.

At screening, biochemistry samples will be collected into a Sarstedt Monovette 4.7 mL z-gel collection tube (or equivalent). 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 (or equivalent). Biochemistry samples will be analysed using the Roche Cobas c6000 analyser series comprising of c501 and e601 modules.

**Urinalysis:** A mid-stream urine sample will be collected into a 20 mL Sterilin tube. Urinalysis will be performed using the Siemens Clinitek 500 analyser or the cobas U6500. Tests included in the Urinalysis screen are SG, pH, leukocytes, nitrites, protein, glucose, ketones, urobilinogen, bilirubin, blood.

In the event that the ‘dipstick’ is positive for leukocytes, nitrites, protein and blood, RBCs, WBCs, epithelial cells, crystals, bacteria and casts will be examined microscopically. Samples of menstruating females may not be sent for microscopy if blood is positive. The microscopy will be performed at [REDACTED] using the cobas u6500 701 module or referred to a local specialist hospital laboratory for analysis. Other positive parameters will be managed as appropriate.

#### **13.4.2. Expired Air Carbon Monoxide (CO)**

Expired CO will be obtained using the Bedfont Micro+ Smokerlyzer<sup>®</sup>. Levels for moderate smokers are defined as >11 ppm, compared to 0-6 ppm for non-smokers.

#### **13.4.3. 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.

#### **13.4.4. 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 the Principal Investigator or designee. 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. The Principal Investigator or designee can examine other body systems if required, at their discretion.

#### **13.7. ECG Measurements**

ECGs will be conducted using a 12-lead ECG at Screening, Day -1 and again prior to discharge from the clinic on Days 4, 13, 17, 21 and 26. 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 Day 25, prior to dosing and continue until approximately 24 hours post dosing.

### 13.9. Subject Diary

All subjects will maintain a daily smoking/medication diary, starting on Day 1 and ending at the Post-Study Visit (6-8 days after the last dose). This will include all time spent in the clinic. Subjects are to record the number of cigarettes smoked in past 24 hours (daily consumption) and actual time they take medication. In addition, subjects are to record any adverse events experienced outside of clinic. Clinic personnel are to review diary when subject is in the clinic and document the number of cigarettes smoked and the actual time medication was taken.

### 13.10. 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 328 mL) is considered acceptable. (See [Table 10](#)).

**Table 10: Summary of Blood Volumes**

Procedure	Visit	No. Samples	Blood Volume per Sample (mL)	Total Blood Volume (mL)
Biochemistry	Screening <sup>a</sup>	1	4.7	4.7
	Day 4, 13, 17, 21, 26	5	4.5	22.5
Haematology	Screening	1	2.7	2.7
	Day 4, 13, 17, 21, 26	5	2.7	13.5
PK Drug Conc. Measurement	Treatment Period	95	3.0	285
<b>Total Blood Volume</b>				<b>328</b>

<sup>a</sup> 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 subjects who have completed all cytisine dosing on Day 1-3, completed >90% cytisine dosing on Days 4-25, and comply with the following criteria:

- Do not have an occurrence of vomiting or severe diarrhoea which renders the concentration profile unreliable;
- Do not use a concomitant medication which renders the concentration profile unreliable;
- 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  $C_{max}$ ;

- Do not violate the protocol (major protocol violation) in a way that may invalidate or bias the PK results.

### **15.2.3. Pharmacodynamic (PD) Analysis Set**

The PD Analysis Set will include all subjects in the PK Analysis Set who have an available baseline result and at least one on-treatment result with regards to urine cotinine, expired air CO or daily cigarette consumption and do not incur a major protocol violation in a way that may invalidate or bias the PD 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 36 subjects will be randomised to the study, with approximately 18 subjects per treatment schedule arm (12 age 18-65 and 6 age >65).

No previous pharmacokinetic studies with cytisine have reported the effects of repeated administration, so no estimates of intra-subject variability of  $C_{max}$ ,  $C_{min}$  or AUC are available. No specific hypothesis testing is planned. Post-hoc analyses may be performed for PK or PD effects that may appear significantly different between the dosage groups or age groups. In general the sample size of 36 should be sufficient to meet the exploratory objectives of the study.

Subjects who withdraw from study at any time after randomisation 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 will be provided to summarise 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  $\pm 2$  minutes from the scheduled sampling time. A deviation greater than  $\pm 2$  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 11: 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 $C_{max}$ .
$C_{min}$	Minimum observed plasma concentration prior to the last daily dose or prior to each scheduled dose change in both treatment group A and B.
$AUC_{0-t}$	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 $\lambda_z$ the apparent terminal elimination rate constant.
%AUC	Residual area or percentage of extrapolated part for the calculation of $AUC_{0-\infty}$ , calculated as $100 * [1 - (AUC_{0-t}/AUC_{0-\infty})]$
$\lambda_z$	Apparent terminal elimination rate constant.
$t_{1/2}$	Apparent terminal elimination half-life, calculated from $\ln 2/\lambda_z$ .

No values of  $\lambda_z$ ,  $AUC_{0-\infty}$ , %AUC and  $t_{1/2}$  will be reported for cases where  $\lambda_z$  cannot be reliably determined.

Pharmacokinetic parameters of cytisine will be derived from the plasma concentrations-time profiles, by using a non-compartmental approach with a ln-linear terminal phase assumption. Actual times of sampling will be used.

$C_{max}$  will be calculated after the first dose and the last dose on Day 1, after the last dose on days 2, 3, 12, 16, 20, 24 and after the morning dose on Day 25.

$C_{min}$  will be derived from pre the first dose plasma values on days 4, 13, 17, 21 and 25.

The  $t_{1/2}$  and AUC ( $AUC_{0-t}$  and  $AUC_{0-\infty}$ ) will be determined after the administration of the final dose of cytisine on Day 25.

Derived plasma pharmacokinetic parameters will be listed and summarised by dose group (1.5 mg, 3.0 mg), and age (18-65, >65 and overall).

*Urine:* The amount excreted in urine over time (Ae) and percent of drug excreted in urine (Ae%) will be derived from Day 1 and Day 25 24 hour urine data for all subjects in the safety analysis set. Derived urine PK parameters will be listed and summarised by treatment dosing (1.5 mg, 3.0 mg), and age (18-65, >65 and overall).

## 15.7. Pharmacokinetic Analysis

Comparisons of  $C_{max}$  and  $C_{min}$  between schedule changes within a dose group will be performed using analysis of variance (ANOVA) on log-transformed data. Point estimates and 90%

confidence intervals (CI) will be constructed for the contrasts between schedule changes which will be back-transformed to give estimates of the ratios of the geometric least squares means (LSmean) and corresponding 90% CI. This analysis will be performed by age group (18-65, >65 and overall) within a dose group.

Comparisons of the Day 25  $C_{max}$ ,  $AUC_{0-t}$  and  $AUC_{0-\infty}$  values between dose groups will be performed using an ANOVA on log-transformed data. Point estimates and 90% confidence intervals (CI) will be constructed for the contrast between the 1.5 mg and 3 mg dose groups which will be back-transformed to provide an estimate of the ratio of the geometric LSmean and corresponding 90% CI. This analysis will be performed by age group (18-65, >65 and overall).

## **15.8. Pharmacodynamic Data**

In order to evaluate any reduction in smoking, expired air monoxide (CO), urine cotinine results and the number of cigarettes smoked daily will be listed and descriptive statistics of absolute and change from baseline results will be tabulated by dose group and age group (18-65, >65 and overall). The mean change from baseline values will also be presented graphically.

## **15.9. Safety Variables and Analyses**

All safety data will be listed using the safety analysis set. Safety summaries will include the number of subjects with adverse events, serious adverse events, and adverse events leading to discontinuation. Adverse events will be assessed by clinical observation and spontaneous reporting by subjects. In addition, number of subjects with clinically significant abnormalities in laboratory values will be summarized.

### **15.9.1. Holter ECGs**

The following ECG parameters will be extracted in triplicate from Holter recordings on Day 1 and Day 25 at 30 and 15 minutes prior to the first dose and 0.5 h, 1 h, 2 h, 3 h, 4 h, 6 h, 8 h, 12 h and 24 h post dose:

- 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 be 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 by dose group and age group (18-65, >65 and overall).

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

For absolute values:

- $QTcF \leq 450$  mSec
- $450 < QTcF \leq 480$  mSec
- $480 < QTcF \leq 500$  mSec
- $QTcF > 500$  mSec.

For change from baseline:

- QTcF increase  $\leq 30$  mSec
- $30 < QTcF$  increase  $\leq 60$  mSec
- QTcF increase  $> 60$  mSec.

Abnormalities in ECGs will be classified in terms of clinical significance. Clinically significant abnormalities will be reported as AEs. The number and percentage of subjects developing new ECG abnormalities will be summarised. The presence of outliers will be explored for heart rate, PR, QRS, QT and QTcF. Details of this analysis will be described in the Statistical Analysis Plan (SAP).

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

#### **15.9.2. 12-Lead ECGs**

12-Lead ECG parameters will be listed with any out of normal range values flagged (“H” -high or “L” -low) as described in the SAP. Descriptive statistics of absolute and change from baseline values at each time point will be tabulated by dose group and age group (18-65, >65 and overall).

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

#### **15.9.3. Vital Signs**

Vital signs parameters will be listed with any out of normal range values flagged (“H” -high or “L” -low). Descriptive statistics of absolute and change from baseline values at each time point will be tabulated by dose group and age group (18-65, >65 and overall).

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

#### **15.9.4. 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, resolution date and resolution 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 dose group, age group and overall.

#### **15.9.5. 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. Descriptive statistics of absolute and change from baseline biochemistry and haematology values at each time point will be tabulated by dose group and age group (18-65, >65 and overall).

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

#### **15.10. 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 centralised 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 organisations. Routine monitoring visits will be conducted to:

1. Assure compliance with the study protocol.
2. Verify that the research facilities, including laboratories and equipment, are adequate to safely and properly conduct the study.
3. 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.
4. 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.



5. Review the subject CRFs and source documents to ensure that reported study data are accurate, complete, and verifiable from source documents.
6. Ensure that adequate records of clinical trial supplies are maintained.
7. Verify that the Investigator and study site personnel are adequately qualified throughout the study.
8. 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:

1. The medical history prior to participation in the study.
2. The basic identifying information, such as demographics, that link the subject's source documents with the CRFs.
3. The results of all diagnostic tests performed, diagnoses made, therapy provided, and any other data on the condition of the subject.
4. The subject's exposure to study treatment.
5. All AEs.
6. The subject's exposure to any concomitant therapy, including dates of administration.
7. All relevant observations and data on the condition of the subject throughout the study.
8. 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 cytosine 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.

## 22. INVESTIGATOR'S AGREEMENT

### Protocol No. ACH-CYT-02

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

## 23. REFERENCES

1. WHO. WHO Report on the Global Tobacco Epidemic, 2008 The MPOWER package. Geneva 2008.
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 Control 2003.
5. Etter J. Cytisine for smoking cessation: A literature review and a meta-analysis. *Archives of Internal Medicine* 2006;166:1553-9.
6. Cahill K, Lindson-Hawley N, Thomas KH, Fanshawe TR, Lancaster T. Nicotine receptor partial agonists for smoking cessation. *The Cochrane database of systematic reviews* 2016:Cd006103.
7. Rigotti NA. Cytisine--a tobacco treatment hiding in plain sight. *The New England journal of medicine* 2014;371:2429-30.
8. Aveyard P, West R. Cytisine and the failure to market and regulate for human health. *Thorax* 2013;68:989.
9. Walker N, Bullen C, Barnes J, et al. Getting cytisine licensed for use world-wide: a call to action. 2016;111:1895-8.
10. 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.
11. Raw M, Anderson P, Batra A, et al. WHO Europe evidence based recommendations on the treatment of tobacco dependence. *Tobacco control* 2002;11:44-6.
12. Heatherton TF, Kozlowski LT, Frecker RC, Fagerstrom KO. The Fagerstrom Test for Nicotine Dependence: a revision of the Fagerstrom Tolerance Questionnaire. *British journal of addiction* 1991;86:1119-27.
13. Heishman SJ, Singleton EG, Pickworth WB. Reliability and validity of a Short Form of the Tobacco Craving Questionnaire. *Nicotine & tobacco research : official journal of the Society for Research on Nicotine and Tobacco* 2008;10:643-51.

## APPENDIX 1. NORMAL RANGES FOR VITAL SIGNS AND ECG PARAMETERS

### Vital Signs:

PARAMETER	Normal Range for ages 18 to 65	Normal Range for ages > 65	Units
Pulse	40 - 110	40 - 110	Beats per Minute (BPM)
Systolic Blood Pressure	90 - 150	90 - 160	mmHg
Diastolic Blood Pressure	50 - 90	50 - 90	mmHg
Oral Temperature	35 - 37.5	35 - 37.5	Degree Celsius

### ECG Parameters:

PARAMETER	Normal Range for all ages	Units
Heart Rate	40 - 110	Beats per Minute (BPM)
PR Interval	120 - 220	msec
QRS width	70 - 120	msec
QT	N/A - should be corrected for heart rate	
QTcB - males	350 - 430	msec
QTcB - females	350 - 450	msec
QTcF - males	350 - 430	msec
QTcF - females	350 - 450	msec

## APPENDIX 2. FAGERSTRÖM NICOTINE DEPENDENCE QUESTIONNAIRE

The Fagerström Nicotine Dependence Questionnaire (FTND) has been shown to be a reliable and valid measure of nicotine dependence<sup>12</sup> and will be administered as outlined in this protocol. Analysis of results is outlined in the study Statistical Analysis Plan.

Question	Response	Points*
How soon after you wake up do you smoke your first cigarette?	Within 5 minutes	3
	6-30 minutes	2
	31-60 minutes	1
	After 60 minutes	0
Do you find it difficult to refrain from smoking in places where it is forbidden (for example in church, at the library, in a cinema, etc.)?	Yes	1
	No	0
Which cigarette would you hate most to give up?	The first one in the morning	1
	All others	0
How many cigarettes/day do you smoke?	10 or less	0
	11-20	1
	21-30	2
	31 or more	3
Do you smoke more frequently during the first hours after waking than during the rest of the day?	Yes	1
	No	0
Do you smoke if you are so ill that you are in bed most of the day?	Yes	1
	No	0

\*Note: The actual questionnaire will not contain the points column, which is provided here to show how responses will be scored for analysis.

### APPENDIX 3. SHORT FORM OF THE TOBACCO CRAVING QUESTIONNAIRE

The template below has been validated as a self-report instrument to measure tobacco craving<sup>13</sup> and will be administered to study subjects as outlined in this protocol. Analysis of results is outlined in the study Statistical Analysis Plan.

Question	Response						
	Strongly Disagree....Strongly Agree						
<i>Factor 1 (emotionality)</i>							
I would be less irritable now if I could smoke.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
If I were smoking now I could think more clearly.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
I could control things better right now if I could smoke.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<i>Factor 2 (expectancy)</i>							
I would enjoy a cigarette right now.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
A cigarette would taste good right now.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Smoking a cigarette would be pleasant.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<i>Factor 3 (compulsivity)</i>							
If I smoked right now, I would not be able to stop.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
I could not stop myself from smoking if I had some cigarettes here.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<i>Factor 4 (purposefulness)</i>							
If I had a lit cigarette in my hand, I probably would smoke it.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
It would be hard to pass up the chance to smoke.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
I could not easily limit how much I smoked right now.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>