Protocol ACH-CYT-08

A Phase I, Double-blind, Randomized, Placebo-controlled, Single Dose-escalation Study to Evaluate the Tolerability and Safety of Cytisine in Adult Smokers

CRO's Protocol Number: BLCL-CYT-01
EudraCT No: 2018-003344-22

07 May 2019

Version 5.0

CONFIDENTIAL

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

Achieve Life Sciences, Inc.
520 Pike Street, Suite 2250
Seattle, Washington, USA 98101
Tel: 425.686.1500 Fax: 425.686.1600

SUMMARY OF CHANGES

OF THIS VERSION (5.0) VERSUS PREVIOUS VERSION (4.0)

Section No.	Section title	Nature of Change
1.	Study Summary – Study Design – Study Treatment Dosing	Three additional dose cohorts (24 mg, 27 mg and 30 mg) were included aiming to reach the stopping criteria.
7.1	Study Design	
1.	Study Summary – Number of Subjects	Up to twenty-four additional participants are planned to be enrolled in the three additional cohorts.
7.2.	Number of Subjects	
1.	Study Summary – Duration of the Study	The information on the duration of the study was updated considering up to 3 additional cohorts.
7.4.	Estimated Duration/ Completion of Study	
1.	Study Summary – Study Procedures	The sample matrix for measurement of cotinine and 3-OH cotinine was changed from plasma to serum because serum is routinely collected, and sample type has no impact on test results.
12.1.1.	Screening visit (Day -28 to Day -1)	
13.3.1.	Routine Laboratory Assessments	
4.3.	Results from Cohorts 1 to 6 (6 mg to 21 mg) of Study ACH-CYT-08	A new section has been created to include the safety and pharmacokinetic results of the previously completed 6 cohorts of the current clinical trial.
10.8.	Study Drug Dosing Schedule	Dosing schedule was updated to include up to three additional cohorts.

SPONSOR SIGNATURE PAGE

Protocol Number	**ACH-CYT-08**
Protocol Title	A Phase I, Double-blind, Randomized, Placebo-controlled, Single Dose-escalation Study to Evaluate the Tolerability and Safety of Cytisine in Adult Smokers
Protocol Version	**Version 5.0**
Protocol Date	07 May 2019

Approvals:

[redacted]

15 MAY 2019
Date

15 MAY 2019
Date

15 MAY 2019
Date

15 May 19
Date

SYNOPSIS

Protocol Number: ACH-CYT-08
Sponsor: Achieve Life Sciences, Inc.
Title of Study: A Phase I, Double-blind, Randomized, Placebo-controlled, Single Dose-escalation Study to Evaluate the Tolerability and Safety of Cytisine in Adult Smokers.
Clinical Phase: Phase 1
Principal Investigator: [redacted]
Clinical Pharmacology Unit: [redacted]
Study Population: Male or nonpregnant female subjects ≥18 years in good general health and who are daily cigarette smokers. The health status will be determined by pre-study medical history, physical examination, vital signs, 12-lead electrocardiogram (ECG), and clinical laboratory tests.
Rationale: (-)-Cytisine is a naturally occurring plant-based alkaloid, isolated from seeds of *Cytisus laburnum* (Golden chain), that is believed to reduce the severity of nicotine withdrawal symptoms while inhibiting nicotine's effects by targeting nicotinic acetylcholine receptors (nAChRs) in the brain. Cytisine, as film-coated tablets of 1.5 mg cytisine, has been used as a smoking cessation drug since the 1960's in Central and Eastern European countries [redacted] manufactured by [redacted] is commercially administered using a 1.5 mg dose in a titration schedule over a 25-day period for a total exposure of 150 mg cytisine. Previous studies dating from decades ago to support use in Central and Eastern Europe, and more recent studies conducted to Good Clinical Practice (GCP), have shown that cytisine can be effective in helping smokers to stop smoking. This GCP conducted, Phase 1 trial is designed to assess the tolerability and safety for a single administered oral dose of cytisine. Since it is difficult to separate the reported adverse effects associated with cytisine treatment versus nicotine withdrawal effects in smokers using cytisine to quit smoking, this Phase 1 study will be performed in smokers who are currently smoking (not trying to quit), are tolerant to the effects of nicotine due to chronic smoking, and will receive only a single dosage of cytisine. The dosage of cytisine will be escalated in separate cohorts until the stopping criteria (based on the occurrence of dose-limiting adverse events) is reached. In addition, plasma cytisine concentrations will be evaluated in order to estimate the cytisine maximum observed plasma concentration (C_{max}) levels associated to the occurrence of dose-limiting adverse events.

Investigational Products:

Test Product: Cytisine 1.5 mg film-coated tablets; manufactured by [redacted],
[redacted]

Control: Placebo film-coated tablets.

Certificates of analysis and batch numbers will be presented in the final Clinical Study Report (CSR).

Objectives:

1. To assess the tolerability and safety of cytisine as a single oral dose.
2. To define the C_{max} levels associated to the occurrence of dose-limiting adverse events.

Study Design:

This is a single-center, double-blind, randomized, placebo-controlled, single dose-escalation, Phase 1 clinical study in adult subjects who are daily smokers, under fasting conditions. Cytisine or placebo will be administered to cohorts of 8 subjects per dose level. The starting dose was 6 mg cytisine (single oral dose) in Cohort 1. Six dose levels were studied under Version 4.0 of Protocol ACH-CYT-08: 6 mg, 9 mg, 12 mg, 15 mg, 18 mg and 21 mg. The study was escalated up to the cytisine dose of 21 mg without showing any of the predefined stopping criteria and remains blinded. Thus, three additional dose levels are pre-planned: 24 mg, 27 mg and 30 mg. Within each dose level, subjects will be randomly assigned to receive a single oral dose of cytisine or placebo in a 3:1 ratio (6 cytisine:2 placebo).

Upon completion of the follow-up assessment for each subject in each cohort, the data will be cleaned and soft-locked and then a safety review will be performed by the Principal Investigator (PI) and the independent Data Safety Monitors (DSMs) on unblinded data. The PI and DSMs will conjointly decide whether dose escalation into the next cohort will be allowed.

Stopping criteria: The dose should not be escalated further if any of the following occurs:

1) A serious adverse event (SAE) that is considered at least possibly related to cytisine in 1 or more subjects;
2) Severe non-serious adverse events that are considered at least possibly related to cytisine in 2 or more subjects within the same cohort;
3) Other safety information considered to pose a risk to subjects.

Selection Criteria:

Inclusion Criteria

1. Free written informed consent prior to any procedure required by the study.
2. Male or female subjects, age ≥18 years, at the time of signing the informed consent.
3. Current daily cigarette smokers (averaging at least 10 cigarettes per day in the past 30 days).
4. Expired air carbon monoxide (CO) ≥10 ppm.
5. Able to swallow multiple tablets at one time.
6. Able to fully understand, comply with all study requirements.

Exclusion criteria:

At screening

1. Known hypersensitivity to cytisine or any of the excipients.
2. Known severe hypersensitivity to any other drug.
3. Positive urinary drugs of abuse screen, determined within 28 days before cytisine/placebo dosing.
4. Positive ethanol breath test.
5. Clinically significant abnormal serum chemistry, hematology, coagulation or urinalysis values within 28 days of randomization (i.e. requiring treatment or monitoring).
6. Clinically significant abnormalities in 12-lead ECG determined after minimum of 5 minutes in supine position within 28 days of randomization (i.e. requiring treatment or further assessment).
7. Body Mass Index (BMI) classification for being underweight (<18.5 kg/m^2) or having ≥Class 2 obesity (≥35 kg/m^2).
8. History of acute myocardial infarction, unstable angina, stroke, cerebrovascular incident, cardiac arrhythmia, or hospitalization for congestive heart failure.
9. Blood pressure ≥160/100 mmHg, measured on the dominant arm, after at least 3 minutes in supine position.
10. Creatinine clearance (CrCl) <80 mL/min (estimated with the Cockroft-Gault equation).
11. Alanine aminotransferase (ALT) or aspartate aminotransferase (AST) >2.0 x the upper limit of normal (ULN).
12. Any inability to comply with study restrictions (See Section 9).
13. Any inability or difficulty in fasting.
14. Difficulty in donating blood on either arm.
15. If woman of childbearing potential, positive result in serum beta-hCG pregnancy test.
16. Women who are breast-feeding.
17. Subjects who do not agree to use acceptable methods of birth control during the study (See Section 9.4).
18. Participation in a clinical study with an investigational drug within the previous 2 months.
19. Participation in more than 2 clinical trials within the previous 12 months.
20. Any other reason that the investigator views the subject should not participate or would be unable to fulfill the requirements for the study.

At admission to each cohort

21. Any recent disease or condition or treatment that, according to the Investigator, would put the subject at undue risk due to study participation.
22. Positive urinary drugs of abuse screen.

23. Positive ethanol breath test.
24. If female of childbearing potential, positive result in urine beta-hCG pregnancy test.
25. Any other reason that the investigator views the subject should not participate or would be unable to fulfill the requirements for the study.

Number of Subjects:

Eight (8) subjects per dose level will be enrolled.

Under ACH-CYT-08 Version 4.0, 48 subjects were enrolled in the previous 6 cohorts. Considering the 3 additional cohorts under ACH-CYT-08 Version 5.0, a total of 72 subjects is estimated for completing enrollment in the study.

Study Treatment Dosing:

According to the randomization schema, individual subjects will receive cytisine or placebo tablets as follows:

Cohort	**Number of 1.5 mg Cytisine Tablets (or placebo)**	**Total Cytisine Dose Administered**
1	4	6.0 mg
2	6	9.0 mg
3	8	12.0 mg
4	10	15.0 mg
5	12	18.0 mg
6	14	21.0 mg
7	16	24.0 mg
8	18	27.0 mg
9	20	30.0 mg

For each additional cohort, the total dose of cytisine will be increased by 3.0 mg (2 additional tablets at 1.5 mg each required for each successive dose level).

Cytisine/placebo will be administered in the morning, orally, with 240 mL of water, after fasting overnight for at least 10 hours.

Diet and Fluids:

Subjects will remain fasted for at least 4 hours following investigational product administration. Except for water given with the investigational product, no fluids will be allowed from 1 hour before until 1 hour after dosing. Standardized meals and snacks identical in all cohorts will be provided not earlier than 4, and at 8±0.5, 12±1, 15±1 and 24±1 hours after dosing.

Safety Assessments: Safety will be evaluated through the assessment of adverse events, ECG, vital signs, and clinical laboratory tests (see Schedule of Study Procedures). Adverse events will be monitored throughout the study. The highest dose-level that resulted in ≤1 cytisine-treated subject within a cohort presenting dose-limiting adverse events will be considered the maximum tolerated dose.
Pharmacokinetic Assessments: Twelve (12) venous blood samples (volume of 6 mL each) will be collected for the determination of plasma concentrations of cytisine, at pre-dose and at predefined time points over 3 hours post dose. Cytisine plasma concentrations will be measured using a previously validated liquid chromatography with tandem mass spectrometry (LC-MS/MS) analytical method. Pharmacokinetic parameters of Cytisine will be estimated with Phoenix™ WinNonlin™ version 8.1 or higher (Certara USA Inc, Princeton, NJ). The following pharmacokinetic parameters will be estimated: C_{max} and time of occurrence of C_{max} (T_{max}).
Volume of Blood Drawn: Subject's total volume of scheduled blood drawn for the pharmacokinetic and clinical laboratory safety assessments will be approximately 90 mL.
Duration of Study: Each cohort will require a minimum of 2 days in the clinical research facilities (subjects will be confined at the clinical research facilities from at least 11 hours before dosing until at least 24 hours post-dose). Each cohort will take 7 days for completion , including a safety review and the final 4-5 day post study follow-up call.
Study Procedures After providing signed informed consent, all subjects will be evaluated for inclusion in the study within a 28-day Screening Period. The Table below provides a summary of required procedures/evaluations during the study.

Schedule of Study Procedures

Assessments	**Screening**	**Treatment Period in Each Cohort**			**Follow-up**
		Admission	**Confinement[1]**	**Discharge[2]**	
Days	-28 to -1	Day 0	Days 1-2	Day 2	Day 5-6[3]
Written informed consent	X				
Demographic data/height/weight	X				
Expired CO levels	X				
Baseline NMR[10]	X				
Medical history, including smoking	X				
Medical history update		X[4]			
Physical examination	X				
Physical examination update		X[4]			
Vital signs (supine blood pressure, pulse rate and body temperature)	X	X	X[5]	X[5]	
Prior and concomitant medication	X	X	X	X	X
12-lead ECG	X			X[6]	
Continuous vital parameters monitoring			X[7]		
Pregnancy test, if WOCBP	X (Serum)	X (Urine)		X (Serum)	
Hematology	X			X	
Biochemistry	X			X	
Estimated creatinine clearance	X				
Coagulation	X				
Urinalysis	X				
Drugs-of-abuse tests in urine	X	X			
Ethanol breath test	X	X			
Verification of eligibility criteria	X	X			
Cytisine/placebo administration			X[8]		
Blood collection for pharmacokinetic analysis			X		
Adverse events monitoring	X[9]	X[9]	X[9]	X[9]	X[9]

WOCBP – Woman of childbearing potential

[1] From at least 11 hours before dosing to at least 24 hours post dose.

[2] At 24 hours post-dose.

[3] A Post-Study Follow-up telephone call will occur 4-5 days after dosing to document any changes in adverse event(s) observed at discharge as well as any new adverse events and concomitant medications taken.

[4] Clinically relevant changes will be reported as adverse events.

[5] Vital signs to be recorded at pre-dose and again at approximately 2, 4 and 6 hours post-dose on Day 1 and at discharge on Day 2.

[6] Repeat 12-lead ECG and assess prior to discharge.

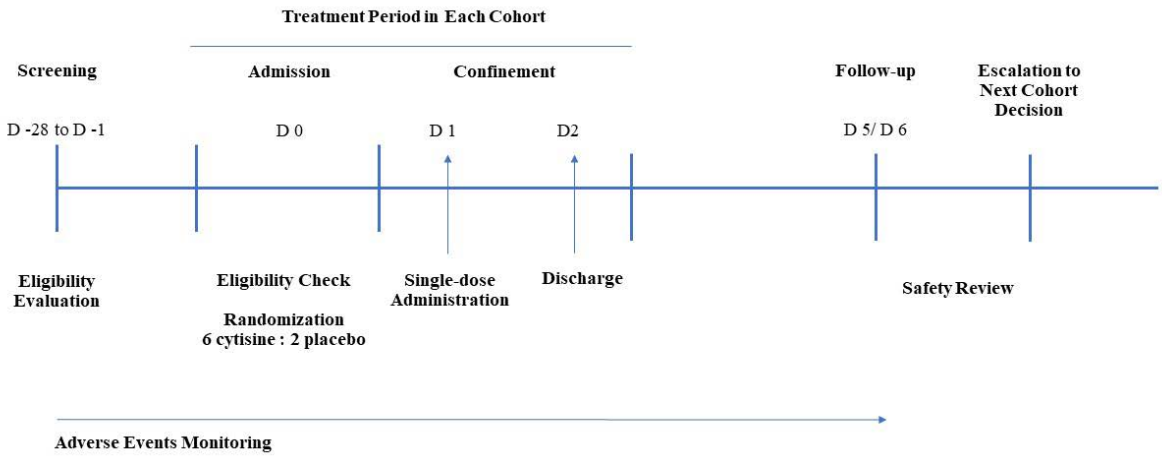
[7] Continuous vital parameters monitoring (lead-II ECG, heart rate, respiratory rate and pulse oximetry) will be performed from pre-dose up to 6 hours post-dose.

[8] Orally, in the morning, with 240 mL of water, after an overnight fasting of at least 10 hours.

[9] Adverse events will be monitored throughout the whole study.

[10] Serum collected for cotinine and 3-OH cotinine to determine baseline nicotine metabolite ratio (NMR).

Study Diagram:



Statistical Considerations:

Analysis Sets

Screening Analysis Set: The Screening Analysis Set is defined as all subjects who give written informed consent and have entered screening. Analyses will be restricted to presentation of baseline data and reasons for non-participation as contrasted to those enrolled and received study drug.

Safety Analysis Set: The Safety Analysis Set is defined as all enrolled subjects who were administered the single dose of study drug.

General Considerations

Summaries of demographics, baseline characteristics and safety endpoints will be provided for the safety population, defined as all subjects who receive a single dose of cytisine or placebo.

Unless otherwise indicated, no statistical comparisons between the cohorts are planned. Summaries will be provided for each cohort. Numeric variables will be summarized using the mean, median, standard deviation, etc. Categorical variables will be summarized with number and percent.

Pharmacokinetic parameters (C_{max} and T_{max}) will be summarized for each cohort using the mean, standard deviation, median, minimum and maximum.

Reporting:

A CSR according to ICH E3 will be prepared on the study. The pharmacokinetic analysis will be integrated in the CSR.

Ethical and Regulatory Compliance:

The study will be conducted in accordance with the approved Protocol and in compliance with the Declaration of Helsinki, ICH Good Clinical Practice, and all applicable laws and regulations.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

ABBREVIATION/ TERM	DEFINITION
ACh	Acetylcholine
ADR	Adverse Drug Reaction
AE	Adverse Event
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
$AUC_{0-\infty}$	Area Under the Curve Extrapolated from Time Zero to Infinity
AUC_{0-t}	Area Under the Curve from Time Zero to Last Sampling Time with Quantifiable Concentrations
AUC_{last}	Area Under the Curve to Last Measured Concentration
BMI	Body Mass Index
[redacted]	[redacted]
CI	Confidence Interval
C_{max}	Maximum Observed Plasma Concentration
CrCl	Creatinine Clearance
CRF	Case Report Form
CSR	Clinical Study Report
DLT	Dose Limiting Toxicity
DSM	Data Safety Monitor
ECG	Electrocardiogram
eCRF	Electronic Case Report Form
FDA	Food and Drug Administration
FSH	High Follicle Stimulating Hormone
GCP	Good Clinical Practice
GD	Gestation Day
GLP	Good Laboratory Practice
GI	Gastrointestinal
HED	Human Equivalent Dose
ICH	International Conference on Harmonization
IMP	Investigational Medicinal Product
[redacted]	[redacted]
IND	Investigational New Drug
LD	Lactation Day
MedDRA	Medical Dictionary for Regulatory Activities
MTD	Maximum Tolerated Dose
NCCIH	US National Center for Complementary and Integrative Health
NMR	Nicotine Metabolite Ratio
NOAEL	No Observed Adverse Effect Level
NRT	Nicotine Replacement Therapy
nAChRs	Nicotinic Acetylcholine Receptors
PK	Pharmacokinetics
PI	Principal Investigator
PSUR	Periodic Safety Update Reports

ABBREVIATION/ TERM	DEFINITION
PT	MedDRA Preferred Term
RR	Relative Risk
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SmPC	Summary of Product Characteristics
SOC	MedDRA System Organ Class
SOP	Standard Operating Procedure
SUSAR	Suspected Unexpected Serious Adverse Reaction
T_{max}	Time to Maximum Observed Concentration
TEAE	Treatment-Emergent Adverse Event
UADR	Unexpected Adverse Drug Reaction
UAE	Unexpected Adverse Event
ULN	Upper Limit of Normal

1. INTRODUCTION AND BACKGROUND

1.1. History of the Investigational Product

(-)-Cytisine (cytisinicline) is a plant-based alkaloid isolated from seeds of *Cytisus laburnum* (Golden chain) and has been used as a smoking cessation drug since the 1960's in Central and Eastern Europe, marketed as [redacted]).[1] It is estimated that over 21 million smokers have been treated with [redacted]. The molecular structure of cytisine has similarities to nicotine and acetylcholine (ACh). Nicotine addiction results, at least in part, from its interaction with neuronal nicotinic acetylcholine receptors (nAChRs). Both cytisine and nicotine compete for these receptors.[2-4] Cytisine has high affinity and specificity for neuronal nicotinic ($\alpha_4\beta_2$) receptors.

Despite its widespread use, cytisine has not been market-approved for use outside Central and Eastern Europe. This may, in part, be explained by [redacted] focus on clinical development of cytisine in its traditional markets where the initial clinical studies were conducted and published, although not in English.[1] Two more recent Phase 3 studies have been conducted and published in 2011 and 2014 in the New England Journal of Medicine.[5,6]

1.2. Nicotine Addiction and Impact on Health

Nicotine is an addictive substance that is rapidly absorbed during cigarette smoking. The drug distributes quickly and is thought to interact with nAChRs in the central nervous system (CNS). Although many smokers attempt to quit smoking, few succeed without pharmacological supportive treatment.

Tobacco smoking contributes to some 7 million premature deaths each year worldwide.[7] It is highly addictive, with more than 95% of unaided attempts at cessation failing by 6 months.[8] Every year that a smoker delays quitting beyond the mid-30s, there is an estimated 3 months reduction in life expectancy.[9]

Although nicotine is primarily responsible for the addictive properties of cigarette smoking, tobacco smoke also contains several hundred gaseous substances and several thousand compounds. There is increasing evidence that the presence of carbon monoxide in cigarette smoke plays a role in cardiovascular disease and that tar is a major factor in respiratory disease and cancer.[10] Nicotine, of which 1 to 3.0 mg is typically absorbed from each cigarette, is a peripheral vasoconstrictor and a sympathomimetic stimulant, which also leads to improvement in mood and attention.

1.3. Treatments for Smoking Cessation

The pharmacotherapies currently available in the US and Western Europe to help smokers quit include nicotine replacement therapy (NRT) and two non-nicotine containing medications: bupropion (Zyban®, Glaxo-SmithKline) and varenicline (Chantix®/Champix®, Pfizer). NRT and bupropion appear to have about equal efficacy.[11,12] Varenicline is more effective than NRT and bupropion. A brief description of each pharmacotherapy is given below.

Nicotine replacement therapy was first introduced in 1978. NRT replaces the nicotine absorbed from cigarettes and helps subjects stop smoking by reducing nicotine cravings, withdrawal symptoms, and mood changes. Available over-the-counter and prescription-only NRT products include: chewing gums, lozenges, transdermal patches, nasal sprays and inhalers. The purpose of

all NRT products is to achieve a sufficient plasma concentration of nicotine (and hence concentration of nicotine at central nAChRs) and so reduce craving for nicotine derived from cigarette smoke. However, the efficacy of NRT (expressed as a pooled Relative Risk (RR) compared with placebo treatment) is limited (overall RR 1.60).[12] In general, the delivery of nicotine from NRT products is relatively slow, and the pharmacokinetic (PK) profile does not resemble that of cigarettes: the time to maximum observed plasma concentration (T_{max}) tends to be longer for NRT, and the maximum observed plasma concentration (C_{max}) is not characterized by a sharp peak, but by a lower and flatter peak. Thus, the smoker does not have the same nicotine experience with NRT products that they do from smoking. Although there do not appear to be safety concerns for NRT usage, the relatively poor response to NRT products as aids to smoking cessation limits their effectiveness as treatment. Side effects of NRT products include: nausea, dizziness, weakness, vomiting, fast or irregular heartbeat, mouth problems with the lozenge or gum, and redness or swelling of the skin around the patch.

Bupropion is one of the most frequently prescribed antidepressants in the US. Zyban® is bupropion re-profiled as a smoking cessation medication in tablet form. Although it reduces nicotine withdrawal symptoms and craving and enhances tobacco cessation, the precise mechanism by which it aids smoking cessation is unknown. The efficacy of bupropion is similar to that of NRT (overall RR 1.62).[11] Although the most commonly observed adverse events consistently associated with the use of bupropion are dry mouth and insomnia, the medication guide for bupropion cites other adverse events and risks related to this product, including seizures, high blood pressure, and allergic reactions. Because bupropion contains the same active substance as the antidepressant Wellbutrin®, users and potential users are urged to talk to their health care professional about risks of treatment with antidepressant medicines.

Varenicline was synthetically developed as a new class of $\alpha_4\beta_2$ nicotine receptor partial agonists, in part, using (-)-cytisine as the structural starting point.[4] Varenicline is a partial agonist at nicotine receptors and acts as a nicotine substitute leading to a gradual decrease in the smoker's physical and psychological dependence on cigarettes. In a recent Cochrane analysis report detailing 6-month abstinence rates, varenicline was found to be more effective than placebo (overall RR=2.25) or bupropion (overall RR=1.39).[13] The most common side effects of varenicline (>5% or twice the rate seen with placebo) include nausea, abnormal (vivid, unusual, or strange) dreams, constipation, flatulence, and vomiting. In addition, the Prescribing Information for varenicline cites other possible serious side effects and risks including new or worsening mental health problems such as changes in behavior, hostility, agitation, or depressed mood. Other warnings and precautions include new or worsening seizures, accidental injury (e.g., trouble driving or operating heavy machinery), cardiovascular events (new or worsening cardiovascular symptoms), allergic hypersensitivity reactions, serious skin reactions, and nausea.

The recently published, international trial (referred as the EAGLES trial[14]) was a large randomized, double-blind, placebo-controlled and active-controlled (nicotine patch) trial comparing varenicline and bupropion at 140 centers in 16 countries between Nov 30, 2011, and Jan 13, 2015. The trial randomized, 8,144 participants. The study did not show a significant increase in neuropsychiatric adverse events attributable to varenicline or bupropion relative to nicotine patch or placebo. Varenicline was more effective than placebo, nicotine patch, and bupropion in helping smokers achieve abstinence, whereas bupropion and nicotine patch were more effective than placebo. Across cohorts, the most frequent adverse events by treatment group were nausea (varenicline, 25%), insomnia (bupropion, 12%), abnormal dreams (nicotine patch, 12%), and headache (placebo, 10%).

In summary, new treatments are still needed that are less costly, more effective, have an improved safety profile, or can more successfully treat individuals who have failed to quit using the above treatments.

2. RECENT EVIDENCE FOR CYTISINE AS A SMOKING CESSATION TREATMENT

Cytisine has recently been evaluated in two large randomized Phase 3 clinical trials that were conducted according to Good Clinical Practice (GCP) in more than 2,000 participants. The overall objectives in these trials were to confirm the efficacy and safety of cytisine according to current clinical development standards and commercial dosing schedule. The cytisine commercial dose is 1.5 mg and administered 6 times a day for the first 3 days, then titrated down on specific days within a 25-day treatment period to only one tablet on Day 25.

The Phase 3 trial (Tabex Smoking Cessation, or TASC[5] trial) was sponsored by the UK Centre for Tobacco Control Studies and evaluated cytisine versus placebo in 740 primarily moderate-to-heavy smokers treated for 25 days in a single center in Warsaw, Poland. The primary outcome measure was sustained, biochemically-verified smoking abstinence for 12 months after the end of treatment. The TASC trial was conceived by Professor Robert West (Department of Epidemiology and Public Health, University College London) and was funded by a grant from the National Prevention Research Initiative, including contributions from Cancer Research UK, Medical Research Council, United Kingdom Department of Health, and others. The results of the TASC trial were published in the *New England Journal of Medicine* in September 2011.[5] The RR for sustained 12-month abstinence was 3.4 for cytisine compared to placebo (8.4% cytisine arm compared to 2.4% placebo arm; P<0.001). The RR for sustained 6-month abstinence was 2.9 (10.0% cytisine arm compared to 3.5% placebo arm; P<0.001). Cytisine was well tolerated with an increase in all-combined gastrointestinal (GI) adverse events (although there was no significant difference in individual GI events between the arms). The safety profile of cytisine was similar to that of a placebo, with no overall difference in the rate of side effects in the two arms.

The second Phase 3 trial (Cytisine As a Smoking Cessation Aid, or CASCAID[6] trial) was conducted by the Health Research Council of New Zealand and was an open-label trial that randomized 1,310 adult daily smokers. Subjects were randomized to receive either cytisine for 25 days or NRT for 8 weeks. Both treatment groups were offered low-intensity telephone behavioral support during trial treatment. The primary outcome measure was continuous self-reported abstinence from smoking one month after a quit date. The RR for continuous one-month abstinence was 1.3 for cytisine (40% cytisine arm compared to 31% in the NRT arm; P<0.001). A secondary outcome included the RR for continuous six-month abstinence which was 1.4 for cytisine (22% cytisine arm compared to 15% in the NRT arm; P=0.002). Cytisine was generally well tolerated, although self-reported adverse events were higher in the cytisine arm compared with the NRT arm. The most frequent adverse events were nausea and vomiting and sleep disorders. The results of the CASCAID trial, which were published in the *New England Journal of Medicine* in December 2014, showed that cytisine was superior to NRT for smoking cessation and, specifically, that cytisine was 1.43 times more likely than nicotine gums or patches to help participants stop smoking and remain non-smokers for six months.[6]

Both studies are described in more detail within the Investigator's Brochure[15].

3. SAFETY OVERVIEW FOR CYTISINE

3.1. Non-Clinical Studies

The US National Center for Complementary and Integrative Health (NCCIH) designated cytisine as "*a drug of national Public Health importance*" and, in collaboration, has sponsored a series of more recent pharmacology and toxicology studies in support of the cytisine Investigational New Drug (IND) and in preparation for formal Phase 3 evaluation. In that regard, NCCIH has sponsored new non-clinical studies to current Good Laboratory Practice (GLP) that have included 28-day repeat dosing for toxicology assessments in rats and dogs as well as two reproductive/developmental studies in rats.

3.1.1. Summary of 28-Day Repeat Toxicology Studies

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 2 mg/kg/day when given orally for 28 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 cytisine is administered for 28 consecutive days.

Daily oral dose administration of cytisine via capsules in Beagle dogs at 0.5, 1, 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 cytisine, 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) should cytisine be administered for 28 consecutive days (NOTE: cytisine is administered for 25 days using a descending dose titration regimen).

Based on the findings in both the 28-day rat and 28-day dog toxicology studies, the established commercial dose of 1.5 mg × 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 total exposure of 18 mg/day for the first 3 days of treatment. This again is below the calculated HED based on all of the studies as shown in Table 1.

Table 1: Calculation of Margin of Safety based on Rat and Dog Toxicology Studies

NOAEL	C_{max} (ng/mL)	AUC_{last} (ng*h/mL)	HED[a] (mg/kg/day)	HED[a] (mg/day)	Human Dose[b]	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	-	-	0.28	20	9 mg/day	~2X

[a] HED = Human Equivalent Dose
[b] Human commercial dose is 9 mg/day for the first 3 days in the 25-day schedule per commercial use.
NOTES: C_{max} 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.

3.1.2. Summary of Reproductive/Developmental Studies

The GLP study titled "Combined Fertility and Embryofetal Development Toxicity Study in Rats with Toxicokinetics" administered oral cytisine once daily to males for 10 weeks (initiating 28 days prior to mating) and to females for 14 days prior to mating, during mating, and through Gestation Day (GD) 17. Reproductive assessment for 10 males/group included sperm assessments with sample collection for evaluation of motility and total sperm count. Females were sacrificed with caesarian section on GD 21 with uterine contents examined and any grossly abnormal cervical, thoracic and abdominal viscera noted. The placenta, amniotic sac, number of live fetuses, number of early or late resorptions, and number of corpora lutea and any abnormalities were also recorded. Fetuses were evaluated for gross, visceral, and skeletal abnormalities. This study has been completed and no treatment-related mortality or clinical signs of toxicity were observed. There was also no evidence of treatment-related effects on mating, cyclicity, impaired fertility or embryofetal development.

The GLP study titled "Pre- and Postnatal Development Toxicity Study in Rats, Including Maternal Function" administered daily cytisine to time-mated females on Gestation Days 6 through Lactation Day (LD) 20. Once daily, during the dosing interval (GD 6 through LD 20), detailed observations were done for each maternal animal at 4 hours post-dosing. Prior to and after the dosing interval, detailed observations were done at the same time that body weights were taken. On LD 0, date of delivery, litter size, and the sex, weight, and observations of individual offspring were recorded. During lactation on LD 4, 7, 14, and 21 (weaning), general development was recorded daily for individual offspring. Maturation phase procedures on LD 22-63 were also evaluated and recorded for development of each offspring. This study has been completed. No treatment-related mortality or clinical signs of toxicity were observed, and no evidence of treatment-related effects on mating, cyclicity, impaired fertility or embryofetal development. A slight treatment-related effect on body weight/body weight gain was seen at the 10.0 mg/kg dose level in both males and females. Systemic exposure was generally higher in females than in males regardless of dose, and C_{max} was slightly higher in females at the 10 mg/kg compared to males at the 10 mg/kg dose level.

An older non-GLP study titled "Embryotoxicity and Teratogenicity Study of Cytisine [redacted] in Rabbits" administered cytisine in sexually mature female rabbits. After fertilization by a male, female rabbits were treated with cytisine daily. Fertilized animals were observed for symptoms of toxicity and weight gain. Female animals were sacrificed by anesthesia on day 28 of pregnancy and were subjected to macroscopic examination of internal organs and Caesarean section of the uterus. After visual examination of fetuses, placentas and fetal resorption remains, fetuses were weighed, examined for external anomalies as well as skeleton and brain structure development were evaluated. Treatment with cytisine did not cause clinical symptoms, death or negative influence on weight gain of pregnant rabbits. Unsuccessful pregnancies, loss of offspring or abortion associated with the effect of cytisine were not found. Increased pre- and post-implantation death of the developing fetus, as well as reduced fetal weight, was not observed. No cases of external or visceral malformations and defects in fetal development were found. Malformations in the development of the skeleton and cranial bones as well as higher incidence rate of delayed ossification and variants in groups exposed to cytisine were also not observed.

Refer to the Investigator's Brochure for more detail on GLP-conducted, non-clinical studies.

3.2. General Safety of Cytisine as a Marketed Product

Cytisine has been marketed by [redacted] in Central and Eastern Europe for over 20 years under the brand name [redacted] is currently marketed in 20 countries and is estimated to have been administered to over 21 million subjects.

[redacted] maintains an independently-audited pharmacovigilance system overseen by a pharmacovigilance Qualified Person (QPPV). Periodic Safety Update Reports (PSURs) have been regularly provided to the relevant European authorities since 2005 (2 years before [redacted] became one of the Member States of the European Union). It is estimated that the cumulative number of exposures to cytisine since 2005, and forming the denominator in the cumulative PSURs is over 15 million cases.

The safety information in the [redacted] Core Data Sheet is regularly reviewed against new PSUR information. Sections 3.2.1 and 3.2.2 summarize the section titled "Undesirable Effects" in the latest [redacted] Core Data Sheet.

3.2.1. Summary of Safety Profile

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=2,844), 1,389 subjects received cytisine. The most commonly reported adverse events in the cytisine group involved the gastro-intestinal system: upper abdominal pain, 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, statistical analysis did not reveal any significant difference in the nervous system adverse events between the cytisine and placebo groups (P=0.12).

3.2.2. Tabulated List of Adverse Reactions

Adverse reactions are listed according to MedDRA system organ class (SOC) and frequency category. Frequency categories are defined using the following convention: very common (>1/10), common (≥1/100 to <1/10), uncommon (≥1/1,000 to <1/100), rare (≥1/10,000 to <1/1,000), very rare (<1/10,000), not known (cannot be estimated from the available data).

Table 2: Adverse Events Reported From the Recent [redacted] Core Data Sheet

System Organ Class	Very Common	Common	Uncommon	Rare	Very rare	Not known
Cardiac disorders						Tachycardia Palpitations
Vascular disorders						Slight increase in blood pressure
Nervous system disorders		Headache[a] Dizziness[a]	Insomnia[a] Drowsiness[a]			Increased irritability
Respiratory, thoracic and mediastinal disorders						Dyspnea
Gastrointestinal disorders		Upper abdominal pain[a] Nausea[a] Dry mouth[a] Dyspepsia[a]	Constipation[a] Diarrhea[a] Vomiting[a]			Changes in taste and appetite Abdominal pain
Musculoskeletal and connective tissue disorders						Myalgia
Metabolism and nutrition disorders						Hyperhidrosis Weight decreased
General disorders and administration site conditions						Chest pain

[a] The frequency was estimated based on data from 6 randomized clinical trials.

Thus, from marketing safety reporting for [redacted] the most frequent adverse effects include: nausea, gastrointestinal symptoms (including abdominal pain, dyspepsia, and dry mouth), sleep disorder, dizziness and headache. Most reported adverse effects appear mainly at the beginning of therapy, are short-lived, mild-to-moderate intensity, and resolved spontaneously. It is not possible to dissociate these effects due to cytisine and those related to nicotine withdrawal in subjects attempting to quit smoking.

Overdoses of cytisine or toxic effects have been reported as nausea, vomiting, mydriasis, tachycardia, and possible breathing paralysis.

4. RECENT STUDIES COMPLETED BY THE SPONSOR

4.1. Preliminary Results from Study ACH-CYT-02 on Safety and Pharmacodynamics of the 3.0 mg Cytisine Dose Compared to the Standard 1.5 mg Dose

ACH-CYT-02 titled "Repeat-Dose Pharmacokinetic and Pharmacodynamic Evaluation of Cytisine in Healthy Smokers" is an open-label, randomized, 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 commercialized 25-day schedule as shown in Table 3.

Table 3: ACH-CYT-02: Dose Timing and Total Daily Dose

Treatment Day	Schedule	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 36 subjects (24 subjects aged 18-65 years and 12 subjects aged >65 years) are planned for enrollment in the study. Twenty-four (24) subjects aged 18-65 have completed the study with safety and pharmacodynamics results presented in this section.

Subject demographics are summarized for the 24 subjects (18-65 years) in Table 4. Twelve (12) (8 male: 4 female) were randomized to 1.5 mg cytisine doses and 12 (7 male: 5 female) were randomized to 3.0 mg cytisine doses. All 12 subjects in the 1.5 mg and all 12 subjects in the 3.0 mg dose groups received cytisine over a 25-day period, with 98.4% and 99.1% of planned doses administered respectively.

Table 4: ACH-CYT-02: Subject Demographics (Aged 18-65)

Parameter	Statistic	18-65 Years		
		1.5 mg Cytisine (N=12)	3.0mg Cytisine (N=12)	Overall (N=12)
Age (yrs)	n	12	12	24
	Mean	34.5	38.6	36.5
	SD	9.60	10.25	9.93
Height (m)	n	12	12	24
	Mean	1.723	1.701	1.712
	SD	0.0763	0.1083	0.0924
Weight (kg)	n	12	12	24
	Mean	84.87	75.53	80.20
	SD	15.115	14.704	15.343
Body Mass Index (kg/m^2)	n	12	12	24
	Mean	28.47	25.98	27.23
	SD	3.922	3.699	3.938
Race:				
- White	n (%)	12 (100.0)	11 (91.7)	23 (95.8)
- Other	n (%)	0 (0.0)	1 (8.3)	1 (4.2)
Gender:				
- Male	n (%)	8 (66.7)	7 (58.3)	15 (62.5)
- Female	n (%)	4 (33.3)	5 (41.7)	9 (37.5)

A total of 42 treatment-emergent adverse events (TEAEs) were reported by 16 (66.7%) subjects during the study; the severity was mild or moderate for all events, the majority of which were related to cytisine (Table 5).

When the number of subjects reporting TEAEs were compared, the same number of subjects reported at least 1 TEAE in each dose group (66.7%). However, there was a slight tendency for a greater number of TEAEs to be reported for 3.0 mg cytisine versus 1.5 mg cytisine (24 events vs 18 events, respectively). There was also a tendency for more subjects reporting moderate events with 3.0 mg cytisine when compared to 1.5 mg cytisine (41.7% vs 16.7% respectively) (Table 5).

There were no SAEs or suspected unexpected serious adverse reactions (SUSAR). All TEAEs were resolved.

Table 5: ACH-CYT-02: Overall Summary of TEAEs by Severity and Relationship – 18 to 65 Years Old (Safety Set)

	18-65 Years		
	1.5 mg Cytisine (N=12)	**3.0 mg Cytisine (N=12)**	**Overall (N=24)**
Number of TEAEs	18	24	42
Mild	13	16	29
Moderate	5	8	13
Severe	0	0	0
Number(%) of Subjects Reporting at Least 1:			
TEAE	8 (66.7)	8 (66.7)	16 (66.7)
Serious TEAE	0 (0.0)	0 (0.0)	0 (0.0)
TEAE Leading to Withdrawal of Study Drug	0 (0.0)	0 (0.0)	0 (0.0)
Number(%) of Subjects with TEAE by Severity:			
Mild	6 (50.0)	3 (25.0)	9 (37.5)
Moderate	2 (16.7)	5 (41.7)	7 (29.2)
Severe	0 (0.0)	0 (0.0)	0 (0.0)
Number(%) of Subjects with TEAE by Relationship to Cytisine:			
Definite	0 (0.0)	0 (0.0)	0 (0.0)
Probable	0 (0.0)	2 (16.7)	2 (8.3)
Possible	4 (33.3)	5 (41.7)	9 (37.5)
Unlikely	2 (16.7)	0 (0.0)	2 (8.3)
Not Related	2 (16.7)	1 (8.3)	3 (12.5)
N/A	0 (0.0)	0 (0.0)	0 (0.0)

A subject with multiple AEs was counted only once at the maximum level of severity or the highest association to cytisine.
AE = adverse events, N/A = not applicable, TEAE = treatment emergent adverse event

Nervous system disorders were the most commonly occurring SOC events (Table 6), with the most commonly occurring TEAE being headache with an overall incidence of 24 headache events in 10 subjects (41.7%). There was a slightly higher number of subjects reporting moderate headaches in the 3.0 mg vs 1.5 mg dose group (33.3% vs 16.7%) (Table 6 footnote). Gastrointestinal disorders were the next most commonly occurring SOC events, with an overall incidence of 2 nausea events in 2 subjects (8%) and 2 constipation events in 2 subjects (8%).

Table 6: ACH-CYT-02: Treatment-Emergent Adverse Events by System Organ Class and Preferred Term – 18 to 65 Years Old (Safety Set)

	Number of Events/Number of Subjects (%)		
SYSTEM ORGAN CLASS: **Preferred Term**	**18-65 Years**		
	1.5 mg Cytisine (N=12)	**3.0 mg Cytisine (N=12)**	**Overall (N=24)**
GASTROINTESTINAL DISORDERS:	3 / 2 (16.7)	5 / 3 (25.0)	8 / 5 (20.8)
Constipation	1 / 1 (8.3)	1 / 1 (8.3)	2 / 2 (8.3)
Flatulence	0 / 0 (0.0)	1 / 1 (8.3)	1 / 1 (4.2)
Nausea	0 / 0 (0.0)	2 / 2 (16.7)	2 / 2 (8.3)
Tooth loss	2 / 1 (8.3)	0 / 0 (0.0)	2 / 1 (4.2)
Toothache	0 / 0 (0.0)	1 / 1 (8.3)[b]	1 / 1 (4.2)
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS:	1 / 1 (8.3)	2 / 2 (16.7)	3 / 3 (12.5)
Chest discomfort	0 / 0 (0.0)	1 / 1 (8.3)	1 / 1 (4.2)
Medical device site reaction	1 / 1 (8.3)	0 / 0 (0.0)	1 / 1 (4.2)
Peripheral swelling	0 / 0 (0.0)	1 / 1 (8.3)	1 / 1 (4.2)
INFECTIONS AND INFESTATIONS:	1 / 1 (8.3)	0 / 0 (0.0)	1 / 1 (4.2)
Rhinitis	1 / 1 (8.3)	0 / 0 (0.0)	1 / 1 (4.2)
INJURY, POISONING AND PROCEDURAL COMPLICATIONS:	2 / 2 (16.7)	0 / 0 (0.0)	2 / 2 (8.3)
Muscle strain	1 / 1 (8.3)	0 / 0 (0.0)	1 / 1 (4.2)
Procedural pain	1 / 1 (8.3)[a]	0 / 0 (0.0)	1 / 1 (4.2)
NERVOUS SYSTEM DISORDERS:	9 / 4 (33.3)	16 / 6 (50.0)	25 /10 (41.7)
Headache	9 / 4 (33.3)[a]	15 / 6 (50.0)[b]	24 /10 (41.7)
Vision blurred	0 / 0 (0.0)	1 / 1 (8.3)[b]	1 / 1 (4.2)
PSYCHIATRIC DISORDERS:	0 / 0 (0.0)	1 / 1 (8.3)	1 / 1 (4.2)
Abnormal dreams	0 / 0 (0.0)	1 / 1 (8.3)	1 / 1 (4.2)
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS:	1 / 1 (8.3)	0 / 0 (0.0)	1 / 1 (4.2)
Oropharyngeal pain	1 / 1 (8.3)	0 / 0 (0.0)	1 / 1 (4.2)
SKIN AND SUBCUTANEOUS TISSUE DISORDERS:	1 / 1 (8.3)	0 / 0 (0.0)	1 / 1 (4.2)
Rash pruritic	1 / 1 (8.3)	0 / 0 (0.0)	1 / 1 (4.2)

[a] Moderate events of headache (16.7% of subjects) and procedural pain (8.3% of subjects).
[b] Moderate events of headache (33.3% of subjects), toothache (8.3% of subjects) and blurred vision (8.3% of subjects).
A subject was counted only once per system organ class and preferred term.

4.2. Results from Study ACH-CYT-01 on the Effect of Food on Cytisine Bioavailability

ACH-CYT-01 titled "A Phase 1 Open Label, Randomized, Two-Way Crossover Study in Healthy Volunteers to Investigate the Effect of Food on the Bioavailability of Cytisine" evaluated 24 healthy volunteer subjects to investigate the effect of food on the bioavailability of cytisine. The primary objective was to compare the bioavailability of cytisine under fed and fasted conditions following administration of 3.0 mg cytisine (2×1.5 mg cytisine tablets).

Eligible subjects received a single-dose of 3.0 mg cytisine under fed and fasted conditions over 2 treatment periods. PK samples (plasma and urine) were collected pre-dose and up to 24 h post-dose (Day 2) during each period (17 plasma and 6 urine samples per period) for the measurement of plasma and urine cytisine concentrations.

The study enrolled 24 subjects (11 males: 13 females) with a median age of 34 years (range 19-53 years). Two of three subjects who experienced emesis during the course of the study (both under fed conditions) were excluded from statistical analysis because emesis occurred at or before 2 times the median T_{max}.

The fasted C_{max} at 29.8 ng/mL was higher than the fed C_{max} at 22.3 ng/mL. The T_{max} under fed conditions (2.75 h) was delayed when compared to the T_{max} under fasted conditions (0.75 h). However, both $AUC_{0-\infty}$ and AUC_{0-t} were comparable under fed (167.5 and 160.2 h.ng/mL) and fasted (172.6 and 165.9 h.ng/mL) conditions, respectively.

Table 7: ACH-CYT-01: Summary of Statistical Analysis of Cytisine Cmax and AUC

Parameter	Geometric LS Mean		Geometric LSMean Ratio (90% CI) fed / fasted
	3.0 mg Cytisine fed (N = 22)	3.0 mg Cytisine fasted (N = 22)	
C_{max} (ng/mL)	22.3	29.8	74.71 (66.39 - 84.08)
$AUC_{0-\infty}$ (h.ng/mL)	167.5	172.6	97.04 (94.20 - 99.98)
AUC_{0-t} (h.ng/mL)	160.2	165.9	96.52 (93.30 - 99.85)

Cytisine showed rapid absorption after oral administration with over 80% of cytisine recoverable in urine within the 24 hours post-dosing.

A total of 22 TEAEs were reported by 10 (41.7%) subjects during the study; the severity was mild for all events, the majority of which were related to cytisine. No serious TEAEs were reported during the study. NOTE: that all subjects enrolled in this study were non-smoking subjects and, thus, were not tolerant to the effects of nicotine as a result of smoking.

The percent of subjects with at least one TEAE was similar under fed and fasted conditions, at 25.0% and 29.2%, respectively. The most commonly reported TEAEs were: dizziness (16.7%), headache (12.5%), vomiting (12.5%) and nausea (8.3%). All were expected events identified in the Investigator's Brochure[15] and the current Summary of Product Characteristics (SmPC)[16] for [redacted] The TEAE profile was similar under fed and fasted conditions, with a similar number of subjects reporting any given event.

There were no clinically significant biochemistry, hematology, urinalysis, vital sign or safety 12 lead electrocardiogram (ECG) values reported during the study.

ECG data extracted from the continuous ECG Holter recordings demonstrated a moderate increase in heart rate but there were no clinically significant changes in QTcF during the study. There was no relationship between cytisine plasma concentration and QTcF.

4.3. Results from Cohorts 1 to 6 (6 mg to 21 mg) of Study ACH-CYT-08

Under the ACH-CYT-8 Protocol Version 4.0, dated 07 Feb 2019, 6 cohorts of 8 adult smokers each were enrolled. In each cohort of 8 subjects, 6 subjects were randomly assigned to cytisine single-dose administration and 2 subjects were assigned to placebo. Cytisine doses were 6 mg (Cohort 1), 9 mg (Cohort 2), 12 mg (Cohort 3), 15 mg (Cohort 4), 18 mg (Cohort 5) and 21 mg (Cohort 6).

Dose escalation to the next cohort was dependent upon a non-blinded interim analysis of the safety data of the preceding cohorts by the Data Monitoring Committee (DMC) composed by the Principal Investigator and two independent Data Safety Monitors (DSMs). The tolerability and safety results were favorable after review of each cohort, with the DMC recommending to proceed with the study protocol as planned. The study remains blinded to the study staff and Sponsor in lieu of continuing the study with additional cohorts.

Based on the tolerability and safety results in each cohort, the study was escalated up to the cytisine dose of 21 mg without showing any of the predefined stopping criteria (based on the occurrence of dose-limiting adverse events).

In accordance with the protocol, any occurrence of adverse events (AEs) as reported by study subjects were monitored throughout the study. Any clinically relevant changes in vital signs, ECG and clinical laboratory safety tests were also reported as AEs.

Most TEAEs were of mild severity and resolved without requiring the use of medication. There were no clinically relevant abnormalities in vital signs, ECG or clinical laboratory safety tests.

There were no severe TEAEs, Serious Adverse Events (SAEs) or any TEAEs leading to discontinuation.

In conclusion, single oral doses of cytisine 6 mg to 21 mg were generally well-tolerated and safe in a population of adult smokers. Although data remain blinded, it appears that the incidence or severity of TEAEs do not yet show an apparent dose-related trend or dose-limiting toxicity and that further dose escalation is warranted to further explore for dose-limiting toxicity of a single cytisine dose.

Preliminary blinded pharmacokinetic data are available for the six previously planned cohorts. Results show that median T_{max} ranged from 0.84 h (6 mg) to 2.00 h (18 mg). Geometric Mean for C_{max} ranged from 68.935 ng/mL (6 mg) to 144.693 ng/mL (21 mg). There was a less than dose-proportional increase of C_{max} (geometric mean) with increasing doses. For a cytisine dose increase in the ratio of 1.50:1.33:1.25:1.20:1.16, C_{max} increased by 1.34:0.99:1.35:1.06:1.10. For an overall dose increase of 3.50, cytisine C_{max} increased 2.10, corresponding to a dose-proportionality factor (DPF) of 0.60.

5. RATIONALE FOR THE STUDY

5.1. Rationale

Previous studies dating decades ago and now more recent GCP studies have shown that cytisine can be effective in helping smokers to stop smoking. Recent Phase 3 trials have shown that cytisine was more effective than both placebo and NRT for smoking cessation with an excellent safety profile that is consistent with the safety profile reported by [redacted] for millions of subjects who have used [redacted]. It is not possible to determine which reported adverse effects associated with cytisine treatment are an effect of the drug itself, due to nicotine withdrawal effects if smoking cessation occurred, or a combination of both. However, the risk/benefit for cytisine appears very acceptable. Since tobacco smoking contributes to some 7 million premature deaths each year worldwide[7] and remains the most important risk to individuals who want to stop smoking, using cytisine as a smoking cessation aid could offer far more benefit with minor risks of treatment when compared to that of continued smoking.

Achieve Life Sciences and Food and Drug Administration (FDA) have discussed development plans for cytisine as a new chemical entity for aiding smoking cessation in the United States, which ultimately led to filing an IND application in 2017. This GCP conducted, Phase 1 trial is designed to assess the tolerability and safety for a single administered oral dose of cytisine. Since it is difficult to separate the reported adverse effects associated with cytisine treatment versus nicotine withdrawal effects in smokers using cytisine to quit smoking, this study will be performed in smokers who are currently smoking (not trying to quit), are tolerant to the effects of nicotine due to smoking, and will receive one single dose of cytisine.

The dosage of cytisine will be escalated in separate cohorts of 8 subjects per dose level until stopping criteria (based on the occurrence of dose-limiting adverse events) are reached. In addition, plasma cytisine concentrations will be evaluated in order to estimate the cytisine C_{max} levels associated to the occurrence of dose-limiting adverse events.

5.2. Benefit/Risk Assessment

This Phase 1 study is being conducted in healthy smokers. The treatment with cytisine for smoking cessation involves starting at high cytisine dosages with a reduction-titration scheme over a 25-day duration, believed to aid in reducing overall withdrawal symptoms and addiction to nicotine. In the present study, cytisine will be administered as a single dose. Therefore, there is no intended clinical treatment or benefit to study subjects.

Cytisine is marketed in some countries of Central and most countries in Eastern Europe as a treatment for smoking cessation. However, it is not yet available for the United States of America (US) and Western Europe (EU) populations. Participation in this study will contribute to the further knowledge and understanding about the drug related to supra-therapeutic doses, thus allowing additional generalization for its commercialization in the US and Western EU.

This study will investigate the tolerability and safety of supra-therapeutic doses of cytisine in healthy smokers. The maximum planned cytisine dose is lower than the human equivalent doses (HED) of the maximum tolerated doses (MTD) and of the no observed adverse effect level (NOAEL) doses in short duration toxicity studies in rats and dogs. Because of this, the risks of the planned doses in the study have been minimized based on pharmacokinetic and safety findings in appropriate animal toxicology studies.

Therefore, it is considered that the risk to the subjects will be minimal, however it is possible that healthy smokers on this study may experience pharmacological adverse effects. To help manage this risk, the study will be conducted at a dedicated Phase I unit with experience in the conduct of human Phase 1 studies with adequate safety monitoring capabilities. Subjects will be monitored during each period for a minimum of 24 hours post-dose and a follow-up assessment 4-5 days after dosing will occur.

To further mitigate the potential risks, the study will follow a dose-escalation design in which single ascending doses of cytisine will be administered to sequential cohorts of participants. The progression to the next cohort will depend on a coordinated assessment of the tolerability and safety results of the preceding cohort by the Study Principal Investigator (PI) and two independent Data Safety Monitors (DSMs) prior to allowing escalation to the next dose level. Appropriate stopping criteria based on adverse events have been predefined and incorporated in the study design (Section 14.2).

Please also refer to the Investigator's Brochure[15], including the Guidance for the Investigator, and the [redacted]s approved Summary of Product Characteristics[16].

6. STUDY OBJECTIVES

1. To assess the tolerability and safety of cytisine as a single oral dose.
2. To define the C_{max} levels associated to the occurrence of dose-limiting adverse events.

7. STUDY DESIGN OVERVIEW

7.1. Study Design

This will be a single-center, double-blind, randomized, placebo-controlled, single dose-escalation, Phase 1 clinical study [redacted]nducted in male or female adults who are ≥18 years age, in good general health and who are current daily cigarette smokers, under fasting conditions.

The study will be comprised of a pre-study screen to define eligible subjects for each cohort in the study. Subjects will be enrolled into dose cohorts as required (8 subjects per cohort), based on individual's availability and site's selection. Upon assignment to a dose cohort, subjects will arrive at the clinical research facilities on Day 0 and will undergo an overnight fast of at least 10 h prior to dosing on Day 1. On the morning of Day 1, subjects will receive a single dose (multiple 1.5 mg tablets to reach the required dose level assigned). Subjects will be confined at the clinical research facilities from at least 11 hours before dosing until at least 24 hours post-dose for PK and safety assessments, being discharged in the morning of Day 2, unless an adverse event requiring confinement is on-going.

Six dose levels were studied under Version 4.0 of Protocol ACH-CYT-08: 6 mg, 9 mg, 12 mg, 15 mg, 18 mg and 21 mg. The starting dose was 6 mg cytisine (single oral dose) in Cohort 1. The study was escalated up to the cytisine dose of 21 mg in Cohort 6 without showing any of the predefined stopping criteria. Thus, three additional dose levels (Cohorts 7 to 9) are pre-planned: 24 mg, 27 mg and 30 mg. Within each dose level, subjects will be randomly assigned to receive a single oral dose of cytisine or placebo in a 3:1 ratio (6 cytisine:2 placebo). Upon completion of the follow-up assessment for each subject in each cohort, the data will be cleaned and soft-locked and

a safety review will be performed by the PI and the independent DSMs on unblinded data. The PI and DSMs will conjointly decide whether dose escalation into the next cohort will be allowed.

Stopping criteria: The dose should not be escalated further if any of the following occurs:

1. A serious adverse event (SAE) that is considered at least possibly related to cytisine in 1 or more subjects;
2. Severe non-serious adverse events that are considered at least possibly related to cytisine in 2 or more subjects within the same cohort;
3. Other safety information considered to pose a risk to subjects.

7.2. Number of Subjects

Eight (8) subjects per dose level will be enrolled.

Under ACH-CYT-08 Version 4.0, 48 subjects were enrolled in the previous 6 cohorts. Considering the 3 additional cohorts under ACH-CYT-08 Version 5.0, a total of 72 subjects is estimated for completing enrollment in the study**.**

7.3. Number of Clinical Sites

This will be a single center clinical trial.

7.4. Estimated Duration/Completion of Study

Each cohort will require a minimum of 2 nights stay in the clinical research facilities (subjects will be confined at the clinical research facilities from at least 11 hours before dosing until at least 24 hours post-dose). Each cohort will take 7 days for completion, including a safety review and the final 4-5 day post study follow-up call.

8. SELECTION OF STUDY POPULATION

Study subjects will be recruited from [redacted] Phase I's pool of healthy volunteers, according to the applicable standard operating procedures (SOPs).

Each potential subject will be provided with an informed consent form that has been reviewed and approved by the [redacted] In accordance with the International Conference on Harmonization (ICH) guidelines on informed consent, the Principal Investigator (or designee) will provide potential subjects with a verbal description of the study including, but not limited to, study purpose, study procedures, restrictions and risks. Potential subjects will be asked to read the consent form and to sign and date it once all of their questions have been answered and they voluntarily agree to participate in the study. Subjects will be assured that they may abandon the study at any time without any prejudice and will receive a copy of the signed informed consent form. In case of amendments to the informed consent form during the study, a renewed written and signed consent must be obtained from each subject still participating in the study.

Upon obtaining signed informed consent, each subject will undergo the screening procedures outlined in Section 12.1.1. A screening log will be maintained by the site and will include documentation for screening failures. Subjects meeting all inclusion/exclusion criteria will then be enrolled onto the study and begin the study procedures.

The following eligibility criteria are designed to select subjects for whom protocol 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.

8.1. Inclusion Criteria

Subjects must meet ALL of the following criteria to be eligible for inclusion into the study:

1. Free written informed consent prior to any procedure required by the study.
2. Male or female subjects, age ≥18 years, at the time of signing the informed consent.
3. Current daily cigarette smokers (averaging at least 10 cigarettes per day in the past 30 days).
4. Expired air carbon monoxide (CO) ≥10 ppm.
5. Able to swallow multiple tablets at one time.
6. Able to fully understand, comply with all study requirements.

8.2. Exclusion Criteria

Subjects meeting ANY of the following exclusion criteria will NOT be eligible for inclusion into the study at screening.

1. Known hypersensitivity to cytisine or any of the excipients.
2. Known severe hypersensitivity to any other drug.
3. Positive urinary drugs of abuse screen, determined within 28 days before cytisine/placebo dosing.
4. Positive ethanol breath test.

5. Clinically significant abnormal serum chemistry, hematology, coagulation or urinalysis values within 28 days of randomization (i.e. requiring treatment or monitoring).
6. Clinically significant abnormalities in 12-lead ECG determined after minimum of 5 minutes in supine position within 28 days of randomization (i.e. requiring treatment or further assessment).
7. Body Mass Index (BMI) classification for being underweight (<18.5 kg/m^2) or having ≥Class 2 obesity (≥35 kg/m^2).
8. History of acute myocardial infarction, unstable angina, stroke, cerebrovascular incident, cardiac arrhythmia, or hospitalization for congestive heart failure.
9. Blood pressure ≥160/100 mmHg, measured on the dominant arm, after at least 3 minutes in supine position.
10. Creatinine clearance (CrCl) <80 mL/min (estimated with the Cockroft-Gault equation).
11. Alanine aminotransferase (ALT) or aspartate aminotransferase (AST) >2.0 x the upper limit of normal (ULN).
12. Any inability to comply with study restrictions (See Section 9)
13. Any inability or difficulty in fasting.
14. Difficulty in donating blood on either arm.
15. If woman of childbearing potential, positive result in serum beta-hCG pregnancy test.
16. Women who are breast-feeding.
17. Subjects who do not agree to use acceptable methods of birth control during the study (See Section 9.4).
18. Participation in a clinical study with an investigational drug within the previous 2 months.
19. Participation in more than 2 clinical trials within the previous 12 months.
20. Any other reason that the investigator views the subject should not participate or would be unable to fulfill the requirements for the study.

Subjects meeting ANY of the following exclusion criteria will NOT be eligible for inclusion into the study at admission to each cohort.

21. Any recent disease or condition or treatment that, according to the Investigator, would put the subject at undue risk due to study participation.
22. Positive urinary drugs of abuse screen.
23. Positive ethanol breath test.
24. If female of childbearing potential, positive result in urine beta-hCG pregnancy test.
25. Any other reason that the investigator views the subject should not participate or would be unable to fulfill the requirements for the study.

9. RESTRICTIONS PRIOR AND DURING THE STUDY

To standardize evaluations and avoid possible negative effects on the measurement of C_{max} and/or on subject's safety, several restrictions are to be adopted as outlined in the following sections.

9.1. Confinement

Each subject will be required to stay 2 nights in the clinical research facilities (arrive at the unit in the afternoon/evening of Day 0, at least 11 hours before dosing, and remain confined until at least 24 hours post-dose on Day 2).

9.2. Diet and Fluid Restrictions

9.2.1. Meal Times

Meals and snacks will be standardized and identical in composition in all cohorts.

A dinner will be served on the evening of Day 0. Subjects will be fasting prior to receiving the single dose of cytisine until lunch. Meals will be as follows:

- Subjects will fast overnight for at least 10 hours before dosing.
- No breakfast on day of dosing.
- Lunch will be served not earlier than 4 hours post-dose.
- Snack will be served at 8±0.5 hours post-dose.
- Dinner will be served at 12±1 hours post-dose.
- Snack will be served at 15±1 hours post-dose.
- Breakfast will be served at 24±1 hours post-dose.

Subjects will be requested to fast for at least 4 hours prior to blood sampling for planned biochemistry tests at screening.

Chewing gum will not be allowed during confinement.

9.2.2. Fluid Intake

No fluids (apart from water taken with dose) are allowed from 1 hour prior to dosing until 1 hour afterwards. Water is then allowed ad libitum.

9.2.3. Alcohol Intake

The consumption of alcohol will be limited to a maximum of 2 units per day from 7 days prior to the study. Alcohol will be avoided completely for at least 2 days prior to dosing and throughout the 24-hour study period.

9.2.4. Caffeine

Food or drink containing caffeine, including coffee, tea, cola, energy drinks or chocolates will be avoided completely for 2 days prior to dosing and while the subjects are confined in the clinical research facilities.

9.2.5. Tobacco

Subjects should agree to abstain from smoking while confined at the clinical research facilities. However, subjects will be allowed to smoke outside the facilities, while accompanied by members of clinical research staff.

9.2.6. Poppy and Sesame Seeds and Cannabinoid Smoke Exposure

Subjects must not eat food containing poppy or sesame seeds for 3 days prior to the admission to study, as consumption can lead to a positive opiate result in the drugs of abuse test. In addition, subjects are advised to avoid indoor spaces contaminated with cannabinoid smoke as exposure could lead to a positive result in the drugs of abuse test.

9.2.7. Grapefruit Juice and Other Restrictions

No food or drink containing pineapple, pomelo, pomegranate, starfruit, grapefruit, cranberry, or Seville oranges (including marmalade and fruit juices), and/or food or drink, sweets, candies or other confectionary containing liquorice will be allowed 7 days prior to the study and while the subjects are confined in the clinical research facilities.

9.3. Exercise

Subjects will be advised to avoid performing strenuous physical exercising within 48 hours prior to blood collection for clinical laboratory tests, because it may affect the results.

9.4. Contraception Requirements

Only nonpregnant females can be admitted to the study and females of childbearing potential must not become pregnant during the study.

A woman is not considered of childbearing potential if she:

- Underwent a permanent sterilization method (e.g. hysterectomy, bilateral salpingectomy and bilateral oophorectomy).
- Is clinically diagnosed infertile.
- Is in a post-menopausal state.

NOTE: A post-menopausal state is defined as no menses for 12 months without an alternative medical cause. A high follicle stimulating hormone (FSH) level in the post-menopausal range may be used to confirm a post-menopausal state in women not using hormonal contraception or hormonal replacement therapy. However, in the absence of 12 months of amenorrhea, a single FSH measurement is insufficient.

Female participants of childbearing potential must accept to take appropriate measures to prevent pregnancy during the study. The acceptable contraceptive measures are the following:

- Abstinence of heterosexual intercourse (when this is in line with preferred and usual lifestyle of the subject).
- Progestogen-only hormonal contraception associated with inhibition of ovulation (oral, injectable, implantable).

- Combined (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation (oral, intravaginal, transdermal).
- Intrauterine device.
- Intrauterine hormone-releasing system.
- Bilateral tubal occlusion.
- Vasectomized partner, who has received medical assessment of the surgical success.

The not acceptable contraceptive measures are the following:

- Progestogen-only oral hormonal contraception, where inhibition of ovulation is not the primary mode of action.
- Male or female condom with or without spermicide.
- Cap, diaphragm or sponge with spermicide.
- Periodic abstinence (e.g., calendar, ovulation, symptothermal, post-ovulation methods).
- Withdrawal (coitus interruptus).

NOTE: Women must use a hormonal contraceptive method from at least 4 weeks prior to admission to study in order to prevent pregnancy, and use the hormonal contraceptive on a stable continuous regimen from at least 2 weeks prior to admission to study until the end of the study to ensure stable plasma hormonal level during the whole study duration. It is recommended that oral contraceptives be taken in the evening.

A male can only be admitted to the study if he is not fertile or agrees to abstain from or to use a condom during sexual intercourse, and not to donate sperm, from study admission until at least 1 month after the last administration of study drug.

10. INVESTIGATIONAL MEDICINAL PRODUCT (IMP)

10.1. Cytisine 1.5 mg Film-Coated Tablets

Cytisine will be supplied by the Sponsor. The cytisine drug product is formulated as a compressed film-coated tablet containing 1.5 mg cytisine in a single tablet. Each tablet is composed of cytisine active substance (as the base) supplied by [redacted] with well-established tablet-forming [redacted]

10.2. Placebo Tablets

Placebo tablets will contain the same excipients as in the cytisine tablet formulation plus an additional [redacted] to match final weight of the cytisine tablet. Placebo tablets will be identical in size, shape, color, and packaging to preserve the double-blind study design.

10.3. Receipt and Storage

The study drug shall be stored in a secure, temperature controlled location and only dispensed by suitably trained staff.

10.4. Administration

Study drug will be supplied as compressed tablets. Tablets should be swallowed whole with water on the morning of Day 1.

10.5. Return/Destruction

All unused study drug should be destroyed by the site unless a prearranged return to depot or Sponsor is necessary.

10.6. Method of Assigning Subjects to Cohort

Eight subjects should be ready for enrollment within each cohort at the same time. The site will determine what subject is enrolled into a given cohort primarily based on subject's availability and ability to escalate into the next cohort.

Using SAS® 9.4 or higher (SAS Institute Inc., Cary, NC, USA), a computer-generated block-wise randomization list adequate for a placebo-controlled studies will be prepared by the Data Management and Analysis Department of [redacted] Eight (8) subjects will be allocated to each treatment cohort. Within each treatment cohort, subjects will be randomly assigned to receive a single oral dose of cytisine or placebo in a 3:1 ratio (6 cytisine:2 placebo).

Once generated, the randomization list will be final and cannot be modified.

Upon confirmation that all entry criteria are met on Day 0 (see Section 12.1.2), subjects will be sequentially assigned a subject number. The subject number will be provided to the unblinded pharmacist on site and will define the treatment to which subjects will be allocated (test or placebo) per the randomization list. The clinical research staff will record study drug (tablet) administration and all related information on the applicable source documents, to allow investigational product accountability.

10.7. Blinding and Code Break Procedures

The randomization list and one set of individual sealed code break envelopes will be generated.

The randomization list will be provided to the unblinded pharmacist for preparation of the double-blind treatment and will indicate subject number and associated treatment. The pharmacist will be the only personnel to have access to the randomization list in order to prepare the drug for administration.

Each code break envelope will be labeled with the subject number and the treatment (cytisine or placebo) will be inside of the sealed envelope. One set of the sealed code break envelopes will be provided to the investigational site for emergency code breaking.

Subjects withdrawn from the study retain their subject number and associated randomization, if already given. New subjects must always be allotted a new subject number; once assigned, subject numbers are never reused within the study site.

10.7.1. Emergency Code Break Procedure:

In case of an emergency, *when knowledge of the investigational product assignment is required for the medical management of an individual subject*, the treatment for that subject may be unblinded. The investigator must notify the Sponsor, DSMs and [redacted] Drug Safety Manager within 24 hours after determining that it is necessary to unblind the treatment assignment.

This documentation must include the name of the individual breaking the blind, the date on which the blind was broken, and a description of the event that led to the unblinding. The investigator must also indicate in source documents and in the Case Report Form (CRF) that the blind was broken and provide the date, time, and reason for breaking the blind. Any adverse event or serious adverse event associated with breaking the blind must be recorded and reported as specified in this protocol.

Monitors will routinely check the integrity of the envelopes that are stored at the site. The envelopes will be collected from the site prior to study close-out and sent to the Sponsor to ensure that they were all intact.

10.7.2. Unblinding for Safety Monitoring to Escalate into Next Cohort

Upon completion of the follow-up assessment for each subject in each cohort, a safety review will be performed by the PI and DSMs on unblinded data. Unblinding of the subjects' codes for the data review will be given by the pharmacist to the PI and DSMs. Only the PI and DSMs will be unblinded for the data review. All other site personnel and subjects will remain blinded until completion of the study, with the above emergency exceptions. Refer to Section 14 for Safety Monitoring.

10.8. Study Drug Dosing Schedule

Study drug will be administered to cohorts of 8 subjects per dose level. Within each dose level, subjects will be randomly assigned to receive a single oral dose of cytisine or placebo in a 3:1 ratio (6 cytisine:2 placebo).

Six dose levels were studied under Version 4.0 of the protocol: 6 mg, 9 mg, 12 mg, 15 mg, 18 mg and 21 mg. The starting dose was 6 mg cytisine (single oral dose) in Cohort 1. The study was escalated up to the cytisine dose of 21 mg in Cohort 6 without showing any of the predefined stopping criteria. Thus, three additional dose levels (Cohorts 7 to 9) under Version 5.0 of the protocol are pre-planned: 24 mg, 27 mg and 30 mg.

10.9. Accountability

The pharmacist or pharmacist designee will maintain records of study drug receipt at the trial site, inventory at the site, dispensing for each subject, and any destruction of unused doses and all empty containers of the used doses for investigational product accountability.

11. PREVIOUS AND CONCOMITANT MEDICATIONS

Subjects will be requested to abstain from taking any medicinal products, including vitamins, food supplements, herbal supplements (including St John's Wort), within 14 days prior to admission until the end-of-study (refer to Section 9.2).

At the discretion of the Investigator, the use of non-study drug medications (either prescription or over-the-counter) may be given if clinically-indicated during the study or if intended for contraception.

If an analgesic is required, acetaminophen (paracetamol), at doses of ≤2 gram/day, is permitted any time during the study except during the overnight fasting prior to dosing. In case a subject is administered another drug its use must be reported and possible impacts on the study outcome must be assessed by the Investigator.

Full details of any new medications must be recorded in the subject's CRF.

12. STUDY PROCEDURES

After providing signed informed consent, all subjects will be evaluated for inclusion in the study within a 28 day Screening Period. Table 8 provides a summary of required study evaluations.

Table 8: Schedule of Study Procedures

Assessments	**Screening**	**Treatment Period in Each Cohort**			**Follow-up**
		Admission	**Confinement**[1]	**Discharge**[2]	
Days	-28 to -1	Day 0	Days 1-2	Day 2	Day 5-6[3]
Written informed consent	X				
Demographic data/height/weight	X				
Expired CO level	X				
Baseline NMR[10]	X				
Medical history, including smoking	X				
Medical history update		X[4]			
Physical examination	X				
Physical examination update		X[4]			
Vital signs (supine blood pressure, pulse rate and body temperature)	X	X	X[5]	X[5]	
Prior and concomitant medication	X	X	X	X	X
12-lead ECG	X			X[6]	
Continuous vital parameters monitoring			X[7]		
Pregnancy test, if WOCBP	X (Serum)	X (Urine)		X (Serum)	
Hematology	X			X	
Biochemistry	X			X	
Estimated creatinine clearance	X				
Coagulation	X				
Urinalysis	X				
Drugs-of-abuse tests in urine	X	X			
Ethanol breath test	X	X			
Verification of eligibility criteria	X	X			
Cytisine/placebo administration			X[8]		
Blood collection for pharmacokinetic analysis			X		
Adverse events monitoring	X[9]	X[9]	X[9]	X[9]	X[9]

WOCBP – Woman of childbearing potential

[1] From at least 11 hours before dosing to at least 24 hours post dose.
[2]At 24 hours post-dose.
[3]A Post-Study Follow-up telephone call will occur 4-5 days after dosing to document any changes in adverse event(s) observed at discharge as well as any new adverse events and concomitant medications taken.
[4]Clinically relevant changes will be reported as adverse events.
[5]Vital signs to be recorded at pre-dose and again at approximately 2, 4 and 6 hours post-dose on Day 1 and at discharge on Day 2.
[6]Repeat 12-lead ECG and assess prior to discharge.
[7]Continuous vital parameters monitoring (lead-II ECG, heart rate, respiratory rate and pulse oximetry) will be performed from pre-dose up to 6 hours post-dose.
[8]Orally, in the morning, with 240 mL of water, after an overnight fasting of at least 10 hours.
[9]Adverse events will be monitored throughout the whole study.
[10]Subjects will be tested for both cotinine and 3-OH cotinine levels for determining their baseline nicotine metabolite ratio (NMR).

12.1. Detailed Description of Study Visits

12.1.1. Screening Visit (Day -28 to Day -1)

All subjects will complete the following screening procedures within 28 days of Day 0 in order to verify required inclusion and exclusion criteria.

1. Written informed consent.
2. Demographic data/height/weight.
3. Medical history data, including smoking history.
4. Physical examination.
5. Vital signs (supine blood pressure, pulse rate and body temperature).
6. 12-lead ECG.
7. Clinical laboratory safety tests (biochemistry, hematology, coagulation, urinalysis, drugs of abuse and serum pregnancy test for females of child bearing potential).
8. Estimated creatinine clearance.
9. Expired CO.
10. Serum for cotinine and 3-OH cotinine testing.
11. Ethanol breath test.
12. Prior and concomitant medication.
13. Adverse events monitoring.
14. Verification of eligibility criteria.

12.1.2. Day 0: Admission

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

On Day 0, the subject will check into the clinical research facilities and the following procedures are to be performed:

1. Medical history update.
2. Physical examination update.
3. Vital signs (supine blood pressure, pulse rate and body temperature).
4. Urine testing for pregnancy in females of child bearing potential.
5. Drugs-of-abuse tests in urine.
6. Ethanol breath test.
7. Prior and concomitant medication.
8. Adverse events monitoring
9. Randomization, after verification of eligibility criteria.

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

All subjects will remain confined in the clinical research facilities overnight.

12.1.3. Day 1: Administration of Cytisine

The following study procedures are to be performed:

1. Pre-dose blood draw for PK to be taken within 30 minutes prior to dosing.
2. Vital signs (supine blood pressure, pulse rate and body temperature) will be recorded within 60 minutes prior to dosing.
3. Cytisine (Per cohort dosing using 1.5 mg tablets)/placebo will be administered after the overnight fast of at least 10 hours and subject will continue fasting until lunch (fasting state). Dosing will be administered with 240 mL (8 fluid ounces) of potable water at room temperature. Except for fluids taken for dosing, no fluids will be allowed from 1 hour before dosing until 1 hour post-dosing. No food should be allowed for at least 4 hours post-dose until lunch is served for all subjects. Meals and snacks will then be provided as outlined in (Section 9.2.1).
4. Blood will be collected and processed for C_{max} assessments as outlined in Section 12.2 after administration of cytisine/placebo (time zero).
5. Vital signs (supine blood pressure, pulse rate and body temperature) will be recorded approximately 2 hours, 4 hours and 6 hours after cytisine administration. Any abnormal values should be followed every 2 hours until returning to baseline levels.
6. Continuous vital parameters monitoring (lead-II ECG, heart rate, respiratory rate and pulse oximetry) will be performed from pre-dose up to 6 hours post-dose.
7. Use of concomitant medications are to be documented.
8. Adverse events monitoring.

All subjects will remain in the clinical research facilities overnight.

12.1.4. Day 2: Discharge at 24 hours post-dose

1. Vital signs (supine blood pressure, pulse rate and body temperature).
2. 12-lead ECG.
3. Clinical laboratory safety tests (hematology, biochemistry and serum pregnancy test for females of child bearing potential).
4. Use of concomitant medications are to be documented.
5. Adverse events monitoring.

Upon final review by the study physician, the subject will be discharged from the clinical research facility at 24 hours post-dosing and will be scheduled for a post-study follow-up telephone call.

12.1.5. Days 4-5 Post-Study Follow-up

A Post-Study Follow-up telephone call will occur 4-5 days after dosing to document any changes in adverse event(s) observed at discharge as well as any new adverse events. If any new adverse events are recorded at this follow-up call, arrangements will be made with the subject to come into the clinical research facility for assessment and then followed until outcome determined. Any concomitant medications must also be documented.

12.1.6. Interim Safety Assessments

Upon completion of the follow-up assessment for each subject in each cohort, the data will be cleaned and soft-locked, and a safety review will be performed by the PI and the two independent DSMs on unblinded data.

The safety review will focus on all the adverse events reported during the study and on any other safety-supporting data requested by the independent data safety monitors, namely safety laboratory, ECG, vital signs, physical examination or medical history.

The PI and DSMs will conjointly decide whether dose escalation into the next cohort will be allowed, based on the cumulative results of safety and tolerability.

12.2. Pharmacokinetic Blood Sampling

12 venous blood samples (volume of 6 mL each, total volume of approximately 72 mL) will be collected into pre-cooled lithium heparin tubes, according to the following schedule:

Table 9: Blood Sampling for Pharmacokinetic Assessments

Blood Sample Number	Time from Dosing
1	Pre-dose (within 30 minutes prior to dosing)
2	00:15
3	00:30
4	00:40
5	00:50
6	01:00
7	01:15
8	01:30
9	01:45
10	02:00
11	02:30
12	03:00

The pre-dose blood samples will be collected within 30 minutes prior to dosing. The post-dose blood samples will be collected within +/-2 minutes 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.

Blood samples for pharmacokinetic analysis will be collected and processed as per the analytical methodology instructions provided by the Bioanalytical Laboratory.

Samples will be labeled with self-adhesive pre-printed labels suitable to withstand freezing temperatures. Labels should bear the following minimum information:

- Labels of blood sample tubes: protocol code, subject number, sample time point, matrix type, and the statement "for Cytisine assay" (statement can be abbreviated if needed).
- Labels of plasma aliquots: protocol code, subject number, sample time point, aliquot series, matrix type, and the statement "for Cytisine assay" (statement can be abbreviated if needed).

At agreed times, samples will be transferred to the bioanalytical laboratory, with each series of aliquots in separate shipments. The samples will be packed in dry ice for transport and no interruption of the freeze cycle is allowed. The temperature in the box during the transport will be monitored. Once the bioanalytical laboratory confirms receipt of the first shipment, the second series of aliquots will be sent, if required. For all shipments, the laboratory should acknowledge in writing the receipt of plasma samples in good condition.

13. SAFETY ASSESSMENTS

Only subjects in good general health are eligible for the study. The healthy status will be determined by pre-study medical history, physical examination, vital signs, 12-lead ECG, hematology, serum chemistry, coagulation and urinalysis.

Creatinine clearance (Cr_{CL}) will be estimated at screening by using the Cockcroft-Gault formula: Cr_{CL} male = [(140-age) x (weight in kg)] / [(serum Cr mg/dL) x (72)]; Cr_{CL} female = 0.85 x (Cr_{CL} male).

Subjects' safety will be monitored during the study. A summary of safety procedures is presented in (Table 8). AEs will be monitored throughout the study.

The Investigator will assess the clinical significance of results of laboratory investigations outside the normal ranges, as defined below in Section 13.1. All changes from screening that are observed at any time during the study and meet the pre-defined requisites for clinical significance will be recorded as AEs.

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

At least one Physician will remain at the clinical site for investigational product administration and until 6 hours post dose, and will remain available on call at all times during the study. The hospital where the clinical research facilities are located has emergency care available on a 24-hour basis.

13.1. Definitions

The definitions adopted are 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 pharmaceutical product and which does not necessarily have to have a causal relationship with the treatment.

An AE can therefore be any unfavorable and unintended sign (including a clinically significant laboratory value or test result), symptom, concomitant illness, accident, or the worsening of an existing medical condition.

Events occurring in subjects in the course of a clinical study during treatment-free periods or on treatment with placebo or a comparative medicine are also to be considered AEs. The definition covers also medication errors and uses outside what is foreseen in the protocol, including misuses and abuse of the product.

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

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

The expected/unexpected status should be evaluated and assessed, by the Sponsor, based on the reference safety information available since expectedness in Pharmacovigilance refers strictly to the information listed or mentioned in the applicable reference safety information and not to event(s) 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 any untoward medical occurrence or effect, that, at any dose:

- Results in death
- Is life-threatening

  NOTE: The term "life threatening" in the scope of "serious adverse events" refers to an occurrence in which the subject was at immediate risk of death from the event as it occurred. It does not include a reaction that, if it had occurred in a more serious form, might have caused death. 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.
- Requires hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability or incapacity
- Results in a congenital abnormality or birth defect
- Is an important medical event which requires medical intervention to prevent one of the above

  NOTE: Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in other situations, such as important medical events that may not be immediately life threatening or result in death or hospitalization but may jeopardize the subject or may require intervention to prevent one of the other outcomes listed in the definition above. These should also usually be considered serious. Examples of such medical events could include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in hospitalization, or the development of drug dependency or drug abuse.

SUSARs: AEs which meet all of the following criteria will be classified as suspected unexpected serious adverse reactions (SUSARs):

- 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 attribution between the event and the medicinal product.

13.2. Recording of Adverse Events

The period of observation for the collection of AEs extends from the time when the subject gives Informed Consent until the end of the study (completion of the Follow-up telephone assessment). For all subjects this period will be extended to follow-up on all on-going AEs after the end of the treatment period until final resolution or until it is medically justifiable to stop further follow-up (e.g. a chronic condition has been reached). Pregnancy in a woman volunteer shall be followed-up to determine its outcome, including spontaneous or voluntary termination, details of birth, presence or absence of any birth defects, congenital anomalies, or maternal and/or newborn complications. If the outcome of the pregnancy meets the criteria for immediate classification of a SAE (e.g. spontaneous or elective abortion–any congenital anomaly detected in an aborted fetus is to be documented, stillbirth, neonatal death, or congenital anomaly), the investigator will report the event by completing a SAE form.

There is no time limit on collection of SAEs that are considered related to the investigational product. If the investigator detects a SAE in a study subject after the end of the period of observation, and considers the event possibly related to prior study treatment or procedures, he or she must communicate this to the Sponsor in order to mutually agree on further measures and appropriate reporting.

The AEs must be documented as soon and as completely as possible on the "Adverse Events" pages into the electronic CRF (eCRF). Follow up information must be entered as soon as available.

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

- Full description of AE.
- Date and time of onset.
- Date and time of resolution.
- Severity of event, to be assessed by an Investigator, or their delegate, in accordance with the definitions below.
- Relationship to study drug to be assessed by an Investigator, or their delegate, in accordance with the definitions below.
- Action taken (dose not changed, not applicable, drug withdrawn).
- Outcome and date of outcome (recovered/resolved, recovering/resolving, not recovered/not resolved, recovered with sequel /resolved with sequel, fatal, unknown) and details of any further follow-up.

Corrections to AE details may be made, but must be dated and signed according to the ICH-GCP requirements. If a suspected diagnosis has been ruled out, the investigator may change the AE term, but should add a comment stating the original suspected diagnosis as well as reasons for change.

AEs that occur during the study should be treated by established standards of care that will protect the life and health of the subjects. If such treatment constitutes a deviation from the protocol, the subjects should be withdrawn from the study and the reason must be documented into the eCRF.

<u>Reporting of Adverse Events</u>

All AEs must be fully validated, evaluated, documented, reported (if applicable) and archived, whether or not they are considered to be drug-related.

AEs defined in the protocol as critical for safety assessments and/or clinically relevant abnormalities (in clinical laboratory safety tests, physical examination, vital signs or ECG) should be reported as AEs within the time frame indicated below, in this protocol.

All SUSARs, i.e. which are not consistent with the known safety profile of the Test product, shall be immediately reported.

13.2.1. Grading Adverse Event Severity

The following grades will be used by an Investigator to describe the severity of all AEs (including clinically-significant laboratory AEs) as shown in Table 10. Only 1 severity grade will be used for each AE (e.g. mild - moderate is not acceptable).

Table 10: Adverse Event Severity

Severity of AE	Definition
Mild	A type of AE that is usually transient and may require only minimal treatment or therapeutic intervention. The event does not generally interfere with usual activities of daily living.
Moderate	A type of AE that is usually alleviated with additional specific therapeutic intervention. The event interferes with usual activities of daily living, causing discomfort but poses no significant or permanent risk of harm to the research participant.
Severe	A type of AE that requires intensive therapeutic intervention. The event interrupts usual activities of daily living, or significantly affects clinical status. The event possesses a significant risk of harm to the research participant and hospitalization may be required.

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

13.2.2. Causality Assessment

The relationship of an AE to the investigational product will be determined and documented by the Investigator, according to best medical judgment as shown in Table 11.

Table 11: Assessment of Attribution to Study Drug

Category	Description
Not Related	The subject did not receive the investigational product or the temporal sequence of the AE onset relative to the administration of the investigational product is not reasonable or the event is clearly related to other factors such as the subject's clinical state, therapeutic intervention or concomitant therapy.
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 investigational product.
Possible	The event follows a reasonable temporal sequence from the time of investigational product 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.

Table 11: Assessment of Attribution to Study Drug

Probable	The event follows a reasonable temporal sequence from the time of study drug administration, and/or follows a known response pattern to the investigational 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 study drug administration, and/or follows a known response pattern to the investigational 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.

13.2.3. Reporting of Serious Adverse Events

[redacted] Phase I will inform the [redacted] Drug Safety Manager, DSMs and Sponsor of all SAEs, SARs or pregnancies occurring within 24 hours of becoming aware of the SAE/SAR or the pregnancy.

Any SAE/SAR or pregnancy will be reported to the [redacted] Drug Safety Manager, DSMs and Sponsor via telephone, fax, e-mail or in person. Any oral communication must be followed by written notes by fax or e-mail. The initial report shall be followed by a detailed written report as soon as possible but no later than 5 days.

The notification should be directed to:

[redacted]

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

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

- Investigator name
- Subject number
- Subject initials
- 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 AE reporting period must be followed clinically until all parameters (including laboratory) have either resolved or been assessed as chronic.

All SUSARs shall be expeditiously reported to [redacted] and Regulatory Agency [redacted]. Expeditiously means that all SUSARs will be reported within 7 days of knowledge (i.e., 7 days after the Sponsor was first aware of the reaction).

The Sponsor will be responsible for expediting report of SUSARs to [redacted], via EudraVigilance, and for providing clinical site the respective copy of the Council for International Organizations of Medical Sciences (CIOMS) form. The Sponsor will be responsible for reporting SUSARs to [redacted]

The clinical site will notify [redacted] of all SAEs (other than SUSARs), after Sponsor's review and approval.

13.3. Laboratory

13.3.1. Routine Laboratory Assessments

Routine laboratory safety samples will be analysed at screening for each subject by the site laboratory. A decision regarding whether a result outside the reference range is of clinical significance or not shall be made by the site investigator and the report will be annotated accordingly. Clinically significant abnormalities occurring during the study will be recorded on the AE page. The reference ranges for the site's laboratory parameters will also be entered in the database and filed in the Investigator site file.

Hematology: Hemoglobin, red blood cells, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), coefficient variation of the red cell distribution width (RDW-CV), total white blood cell (WBC) count, neutrophils, lymphocytes, monocytes, eosinophils, basophils and platelets.

Chemistry: Total protein, albumin, direct bilirubin, indirect bilirubin, total bilirubin, alanine aminotransferase (SGPT or ALT), aspartate aminotransferase (SGOT or AST), gamma-glutamyltransferase (GGT), lactate dehydrogenase (LDH), alkaline phosphatase (ALP), glucose,

total cholesterol, triglycerides, sodium, potassium, calcium, creatine phosphokinase (CPK), creatinine, estimated creatinine clearance, uric acid and urea.

Coagulation: prothrombin rate, prothrombin time - international normalized ratio (INR) and activated partial thromboplastin time (aPTT).

Urinalysis: pH, specific gravity, protein, hemoglobin, glucose, ketones, bilirubin, nitrites, urobilinogen and microscopy.

Urine test for drugs-of-abuse: cannabinoids, opiates, cocaine, amphetamines, and benzodiazepines.

Expired CO will be obtained using a calibrated instrument provided and maintained by the clinical site. Cotinine and 3-OH cotinine will be measured in serum for determining the baseline NMR. Ethanol will be measured via a breath test. Pregnancy testing will be performed using both serum and urine beta-human chorionic gonadotropin (beta-hCG) pregnancy tests.

13.4. Vital signs and electrocardiogram

Systolic/diastolic blood pressure, pulse rate, and temperature measurements will be recorded in a supine position. Body weight will also be recorded. Height is to be recorded at Screening Visit for BMI calculation.

12-lead ECG will be recorded in supine position.

Post-dose vital signs and ECG recordings will be taken within ±15 minutes of scheduled time. When ECG or vital signs recordings coincide with a blood draw, they should preferably be performed before the blood collection.

Lead-II continuous (real time) ECG monitoring will be performed from pre-dose to at least 6 hours post-dose.

13.5. Physical Examination

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

14. SAFETY MONITORING

14.1. Independent Data Safety Monitors

Safety monitoring will be performed by two independent DSMs who will be appointed for this study. The DSMs will be M.D.s experienced in the treatment of adult smokers. The primary responsibility of the DSMs will be to monitor for any unexpected safety risk for subjects on the protocol. The DSMs will:

- Review all SUSARs and SAEs. The [redacted]l Drug Safety Manager will provide each DSM with a copy of any unexpected study drug-related SAE Report Form within 7 business days of receipt. The [redacted] Drug Safety Manager (or designee) will

also provide the DSMs with copies of all expedited SAE reports submitted to regulatory agencies.

- Review overall safety for the study (e.g. severe adverse events and other possible dose-limiting adverse events) with the Principal Investigator prior to doses being escalated into the next cohort. The DSMs have the authority to suspend dosing in any cohort at any time.
- The DSMs must review and approve any replacement of a subject within a cohort to assure that the reason for the subject withdrawal did not involve a potential safety issue or hidden dose-limiting adverse event. A subject who enrolled but then withdrew prior to receiving any study treatment may be replaced without DSMs approval.

14.2. Stopping Criteria

Upon completion of the follow-up assessment for each subject in each cohort, the data will be cleaned and soft-locked and a safety review will be performed by the PI and the DSMs on unblinded data. Dose escalation to the next level will be conjointly decided by the PI and the DSMs.

The dose should not be escalated further if any of the following occurs:

1. A serious adverse event (SAE) that is considered at least possibly related to cytisine in 1 or more subjects;
2. Severe non-serious adverse events that are considered at least possibly related to cytisine in 2 or more subjects within the same cohort;
3. Other safety information considered to pose a risk to subjects.

The maximum tolerated dose (MTD) will be defined as one dose-level below which stopping criteria has been observed.

15. STATISTICAL CONSIDERATIONS

15.1. Sample Size

The sample size of eight subjects per cohort is based on what is reasonable and feasible for a Phase 1 dose-escalation study.

15.2. Analysis Sets

Screening Analysis Set: The Screening Analysis Set is defined as all subjects who give written informed consent and have entered screening. Analyses will be restricted to presentation of baseline data and reasons for non-participation as contrasted to those enrolled and received study drug.

Safety Analysis Set: The Safety Analysis Set is defined as all enrolled subjects who were administered the single dose of study drug.

15.3. Safety Assessments

Clinically significant abnormalities will be reported as AEs.

Adverse events will be coded using the MedDRA dictionary version 21.0 or higher. Coding includes SOC and preferred term (PT). The following information recorded or computed is used for the description of the AEs: reported Term; SOC and PT by MedDRA coding; onset date, onset time, offset date and offset time; seriousness; severity (intensity); relationship (causality); action taken; outcome.

The profiles of adverse events for the arms with regard to incidences of TEAEs will be assessed. TEAEs are defined as AEs not present prior to administration of investigational product, or AEs present before administration of investigational product that worsen after the subject receives the dose of investigational product. A separate listing of SAEs will be presented, if applicable. Frequencies of TEAEs will be presented by SOC and PT, by Test product and placebo and overall.

Dose-limiting adverse event determinations are described in Section 14.2 and safety summaries are described in the Statistical Analysis Plan (SAP).

15.4. General Considerations

Summaries of demographics, baseline characteristics and safety endpoints will be provided for the safety population, defined as all subjects who receive a single dose of cytisine or placebo.

Unless otherwise indicated, no statistical comparisons between the cohorts are planned. Summaries will be provided for each cohort. Numeric variables will be summarized using the mean, median, standard deviation, etc. Categorical variables will be summarized with number and percent.

Assessments for trends in dose-limiting adverse events or C_{max} levels in relationship to BMI categories/levels and baseline NMR may be explored.

Unless otherwise indicated, safety summaries will be produced using SAS® software, version 9.4 or higher.

15.5. Pharmacokinetic Parameters

Pharmacokinetic parameters (C_{max} and T_{max}) will be summarized for each cohort using the mean, standard deviation, median, minimum and maximum.

C_{max} will be calculated as the maximum observed plasma concentration (ng/mL) and summarized by mean (90% CI).

T_{max} will be the time to occurrence of maximum plasma concentration (h) and summarized using the median with minimum and maximum.

16. REGULATORY AND ETHICS CONSIDERATIONS

16.1. Independent Ethics Committee

This protocol, informed consent form, details of subject compensations and any subject recruiting materials will be submitted to [redacted]) for evaluation and approval, prior to the start of the study.

The study will not start until the [redacted] has approved the documents. The letter of the [redacted] approval will be appended to the final CSR.

All amendments or revisions to the protocol must undergo review by [redacted].

A copy of the [redacted] approval letter must be forwarded to the Sponsor or Sponsor's representative before the study is implemented. The approval letter must clearly state the protocol title and version that was reviewed, as well as any associated documents. The Investigator also must forward copies of subsequent amendment approval letters to the Sponsor upon receipt.

16.2. Ethical Conduct of the Study

This trial will be conducted in accordance with the current version of the Declaration of Helsinki, as well as the ICH Guidelines on GCP, and applicable laws and regulations.

16.3. Informed Consent

The informed consent forms used for the study must comply with the Declaration of Helsinki and its updates and the ICH GCP and must have been approved by the Sponsor or Sponsor's representatives. The Investigator or an authorized associate, who must be a physician, must explain the nature of the study and the treatment in such a manner that the subject is aware of his/her rights and responsibilities, as well as potential benefits and risks. The Investigator is also responsible for answering any questions the subject may have throughout the study and sharing any new information, in a timely manner, that may be relevant to the subject's willingness to continue his/her participation in the trial.

Subjects must also be informed that participation is voluntary and that they may withdraw from the study at any time, without prejudice to their current or future care. Documentation of the discussion and the date of informed consent should be recorded in the subject's medical record or a study/clinic chart. Once all of their questions have been answered and they have voluntarily agreed to participate in the study, subjects will be asked to sign and date the Informed Consent Form.

Subjects must give informed consent in writing prior to the performance of any protocol-specific procedure. A copy of the signed Informed Consent Form will be provided to the subject.

In case of amendments to the Informed Consent Form during the study, a renewed written and signed consent must be obtained from each subject still participating in the study.

16.4. Review and Approval by Regulatory Authorities

The study will be submitted to the review and approval of [redacted] according to rules in force. The letter of the [redacted] approval will be appended to the final report.

16.5. Insurance, Indemnity and Compensation

It is the Sponsor's responsibility to guarantee sufficient insurance coverage for this study should any serious events or deaths result directly from the execution of the present protocol.

The present article is not to be interpreted as engaging the Sponsor's responsibility in the event of fault or negligence of the subjects, investigators, or any persons or employees under the control of [redacted]

16.6. Subject Confidentiality

The Investigator must attempt to assure that the subjects' confidentiality will be maintained within the limit of the law. Subjects will be identified by subject screening and/or randomization number on all documents submitted to the Sponsor. Subjects will not be identified by name.

All records will be kept in a secure place in the clinical research site. Computer data entry and data review programs will be done using subject screening and/or randomization numbers only. Clinical information will not be released without written permission of the subject, as outlined in the subject consent form.

The Investigator must maintain a log of subject names and identification codes.

17. DOCUMENTATION

17.1. Study File and Site Documents

Prior to the activation of the study, at a minimum, the following items must be received by the Sponsor from the site:

1. Confidential Disclosure Agreement
2. Signed protocol, and amendment(s) page(s)
3. The Principal Investigator's curriculum vitae and current medical license.
4. Signed Clinical Study Agreement
5. Signed Financial Disclosure Form from the relevant site personnel
6. [redacted] written approval for the protocol, amendment(s), Informed Consent Form
7. FDA Form 1572

17.2. Study Documents Supplied by the Sponsor

The Sponsor will supply the investigator with the following items:

1. Current version of the Investigator's Brochure
2. Current version of study protocol
3. Master CRF
4. Informed Consent Form template

17.3. Maintenance and Retention of Records

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

Investigators will be instructed to retain all study records required by the Sponsor and regulatory authorities in a secure and safe facility with limited access for one of the following time periods based on notification from the Sponsor:

1. For a period of 25 years after completion or discontinuation of the trial, or
2. For a period of at least 2 years from the **last** marketing approval worldwide,
3. Or a period of at least 2 years after discontinuation of clinical development of the investigational product as confirmed by the Sponsor.

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.

18. ADMINISTRATIVE PROCEDURES

18.1. Sponsor Responsibilities

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

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 destruction or 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 drug.
5. Review the subject CRFs and source documents to ensure that reported study data are accurate, complete, and verifiable from source documents.
6. Verify that the Investigator and study site personnel are adequately qualified throughout the study.
7. Verify that the safety information and amendments are submitted to [redacted].

18.2. 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.

18.3. Regulatory Compliance

The study will be conducted and submitted to quality control to assure data integrity, in accordance with applicable [redacted] SOPs. These SOPs require quality control on the following processes: medical writing, source documents and eCRF data entry completion and accuracy, data management and study reporting.

The monitoring will be done at the trial site by an independent study monitor. The study monitor will be allowed to check all study documents. It will be his/her responsibility to verify the adherence to the protocol and the completeness, consistency and accuracy of the clinical data.

Quality Assurance representatives from the Sponsor or their delegate, 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 Guidelines.

The Principal Investigator will allow the verification of records (source data) during audits by Sponsor or inspections by national or foreign regulatory bodies, in case such authorities require a regulatory inspection.

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

18.4. Protocol Modification/Premature Termination

All protocol amendments must be written and approved by the Sponsor. [redacted] will review and approve amendments prior to their implementation in the study. [redacted] and [redacted] approval need not be obtained prior to removal of an immediate hazard to subjects.

Deviations from the pre-specified dose escalation and decision-making criteria will be submitted to Ethics Committee [redacted] and Competent Authority [redacted] as substantial amendment(s) for its approval, prior to implementation.

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

The Principal Investigator may terminate participation at his/her site at any time but must provide all study data for subjects randomized.

Following a decision of temporary suspension or discontinuation, it is a responsibility of the Principal Investigator to inform the study subjects and [redacted] stating the reasons for premature termination. The Sponsor shall be responsible for expedited reporting and/or notification to the FDA, as applicable.

18.5. Final Study Report

At the end of the study, an integrated CSR will be prepared following the European electronic Common Technical Document (e-CTD) format specifications and in accordance with the applicable SOPs. The pharmacokinetic analysis will be included in the integrated CSR.

18.6. 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.

Protocol No. ACH-CYT-08, Version 5.0

19. INVESTIGATOR'S AGREEMENT

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 non-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/destruction forms and all other information collected during the study) in accordance with the current GCP and local regulations.

[redacted]	
Site Principal Investigator's name	Sponsor's Representative's name
Signature	Signature
Date **(ddMMMyyyy)**	Date **(ddMMMyyyy)**

[redacted]

20. REFERENCES

1. Etter J. Cytisine for smoking cessation: A literature review and a meta-analysis. Archives of Internal Medicine 2006;166:1553-9.

2. Papke RL, Heinemann SF. Partial agonist properties of cytisine on neuronal nicotinic receptors containing the beta 2 subunit. Molecular pharmacology 1994;45:142-9.

3. Parker MJ, Beck A, Luetje CW. Neuronal nicotinic receptor beta2 and beta4 subunits confer large differences in agonist binding affinity. Molecular pharmacology 1998;54:1132-9.

4. Coe JW, Brooks PR, Vetelino MG, et al. Varenicline: an alpha4beta2 nicotinic receptor partial agonist for smoking cessation. Journal of medicinal chemistry 2005;48:3474-7.

5. West R, Zatonski W, Cedzynska M, et al. Placebo-Controlled Trial of Cytisine for Smoking Cessation. New England Journal of Medicine 2011;365:1193-200.

6. Walker N, Howe C, Glover M, et al. Cytisine versus nicotine for smoking cessation. The New England journal of medicine 2014;371:2353-62.

7. World Health Organization. WHO report on the global tobacoo epidemic, 2017: monitoring tobacco use and prevention policies. Geneva 2017.

8. 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.

9. 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.

10. Foulds J, Burke M, Steinberg M, Williams JM, Ziedonis DM. Advances in pharmacotherapy for tobacco dependence. Expert opinion on emerging drugs 2004;9:39-53.

11. Hughes JR, Stead LF, Hartmann-Boyce J, Cahill K, Lancaster T. Antidepressants for smoking cessation. The Cochrane database of systematic reviews 2014:Cd000031.

12. Stead LF, Perera R, Bullen C, et al. Nicotine replacement therapy for smoking cessation. The Cochrane database of systematic reviews 2012;11:Cd000146.

13. 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.

14. Anthenelli RM, Benowitz NL, West R, et al. Neuropsychiatric safety and efficacy of varenicline, bupropion, and nicotine patch in smokers with and without psychiatric disorders (EAGLES): a double-blind, randomised, placebo-controlled clinical trial. The Lancet 2016;387:2507-20.

15. Cytisine 1.5 mg Film-coated Tablets Investigator's Brochure, Version 6.0, 15FEB2019

16. [redacted] dated January 2018.