- **Protocol number:** Z7244J01
- **Document title:** Phase I Study to Evaluate Pharmacokinetics, Safety, and Tolerability of Single and Multiple i.v. Doses of N-acetylcysteine (NAC) in Chinese Healthy Volunteers
- **NCT number:** NCT03881163
- **Version number:** 3.0
- **Date of the document:** 03 December 2019

16.1 Study Information

16.1.1 Protocol and Protocol Amendments

The following documents are included:

- Clinical Study Protocol, Version 1.0, dated 30 Aug 2018
- Clinical Study Protocol, Version 2.0, dated 19 Dec 2018
- Clinical Study Protocol, Version 3.0, dated 03 Dec 2019

CLINICAL STUDY PROTOCOL

Sponsor code Z7244J01

Phase I study to evaluate pharmacokinetics, safety and tolerability of single and multiple i.v. doses of N-acetylcysteine (NAC) in Chinese healthy volunteers

Single and multiple dose, single centre, open-label, one-way, pharmacokinetics, safety and tolerability clinical trial

Test formulation:	NAC 300 mg/ 3 mL solution for injection, Zambon S.p.A., Italy.
Sponsor:	Zambon S.p.A., via Lillo del Duca 10, I-20091 Bresso, Italy Phone: +39.02.66.52.41 Fax: +39.02.66.50.14.92
Investigator:	"Name Surname", MD - Principal investigator XXXXXXX, Phase I Unit, "Address" China Phone: +xx.xx.xxxxx Fax: +xx.xx.xxxxx Email: xxx@xxxx
Development phase:	I
Version and date:	Final version 1.0, 30 August 2018

This study will be conducted in accordance with the current version of Good Clinical Practice (GCP), ICH topic E6 (R2 and CFDA GCP)

This document comprises 63 pages

1 PROTOCOL APPROVAL

1. SPONSOR

Zambon S.p.A., Italy

Sponsor's representative

PPD

PPD PPD

Date Signature

2. INVESTIGATOR

Principal investigator
I have read this protocol and agree to conduct this study in accordance with all the stipulations of the protocol and in accordance with the Declaration of Helsinki, the current revision of Good Clinical Practice (GCP), ICH topic E6 (R2), and the applicable local law requirements.

Name Surname, MD

PPD PPD

Date Signature

2 STUDY SYNOPSIS

Title: Phase I study to evaluate pharmacokinetics, safety and tolerability of single and multiple i.v. doses of N-acetylcysteine (NAC) in Chinese healthy volunteers

Protocol number: Z7244J01

Clinical phase: Phase I

Study design: Single and multiple dose, single centre, open-label, one-way, pharmacokinetics, safety and tolerability clinical trial

Planned nr. of centres / countries: 1/China

Investigator and centre: *Principal investigator*: "Name Surname", MD, "Institution name", Phase I Unit, "Address"…., China

Investigational medicinal product (IMP):
Test product: NAC, 300 mg/ 3 mL solution for injection, Zambon S.p.A., Italy

Dose regimen:
Two ampoules (300 + 300 mg) corresponding to a total dose of 600 mg of NAC diluted in 10 mL of NaCl 0.9% saline solution, will be administered by a 5-minute intravenous (i.v.) infusion.
Single dose:
One (1) dose of the investigational product will be administered under fasting conditions on day 1 at 08:00 ±1 h
Multiple dose regime:
Five (5) doses of the investigational product will be administered twice a day (b.i.d.) on days 4 and 5 at 08:00 ±1 h and 20:00 ±1 h and one dose will be administered on day 6 at 08:00 ±1.

Objective: To evaluate the NAC pharmacokinetics, safety and tolerability after single and multiple dose i.v. administration.

End-points:
Primary end-point:
- To evaluate pharmacokinetic parameters of NAC in plasma after single and multiple dose administration of the investigational product.

Secondary end-points:
- To collect safety and tolerability data after single and multiple dose administration of the investigational product.

Study variables:
Primary variables:
After single dose:
- C_{max}: maximum NAC plasma concentration
- t_{max}: time to achieve C_{max}
- k_{el}: terminal elimination rate constant, calculated, if feasible, by log-linear regression using at least 3 points
- $t_{1/2}$: NAC half-life, calculated, if feasible, as $ln2/k_{el}$
- $AUC_{(0-t)}$: area under the concentration-time curve from single dose to the last observed concentration time t, calculated with the linear up/log down trapezoidal method
- $AUC_{(0-\infty)}$: area under the concentration-time curve extrapolated to infinity, calculated, if feasible, as $AUC_{(0-t)} + C_t/k_{el}$, where C_t is the last measurable drug concentration
- $AUC_{(0-12)}$: area under the concentration-time curve at steady-state in the tau interval (from single dose to 12 h post-dose), calculated with the linear up/log down trapezoidal method
- V_d: volume of distribution associated with the terminal slope, calculated, if feasible, as $Dose/(AUC_{(0-\infty)}*k_{el})$
- CL_t: total body clearance, calculated, if feasible, as $Dose/ AUC_{(0-\infty)}$
- $Ae_{(0-t)}$: total amount of NAC excreted in urine from single dose up to 32 h
- $Fe_{(0-t)}$: total fraction of NAC dose excreted in urine from single dose up to 32 h
- CL_r: renal clearance, calculated, if feasible, as $Ae_{(0-t)}/ AUC_{(0-\infty)}$

STUDY SYNOPSIS (cont.)

Study variables (continued):
After multiple dose:

- C_{ss_max}: maximum NAC plasma concentration at steady-state
- t_{ss_max}: time to achieve C_{ss_max}
- C_{ss_min}: trough NAC plasma concentration at steady-state, measured as concentration at t=12 h
- $AUCss_{(0-t)}$: area under the concentration-time curve at steady-state from the last multiple dose to the last observed concentration time t, calculated with the linear up/log down trapezoidal method
- $AUCss_{(0-12)}$: area under the concentration-time curve at steady-state in the tau interval (from the last multiple dose to 12 h post-dose), calculated with the linear up/log down trapezoidal method
- C_{ss_av}: average NAC plasma concentration at steady-state, calculated as $AUCss_{(0-12)}$ /tau
- R: accumulation ratio, calculated as $AUCss_{(0-12)}/ AUC_{(0-12)}$
- DF%: degree of fluctuation over one dosing interval at steady-state, calculated as $(C_{ss\_max} - C_{ss\_min})/ C_{ss\_av}*100$
- $Aess_{(0-t)}$: total amount of NAC excreted in urine from the last multiple dose to 32 h at steady-state

Secondary variables:

- Treatment emergent adverse events (TEAEs), vital signs (blood pressure, heart rate), body weight, physical examinations, clinical laboratory parameters.

Analytics: total NAC will be determined in plasma and urine at a certified bioanalytical laboratory to be designated, using a LC-MS/MS validated method with a Lower Quantification Limit (LQL) of 10 ng/mL. Analytical facilities and procedures are in compliance with the general principles of GLP regulations

Sample size: Twenty-four (24) healthy male and female Chinese volunteers will be included in the study. Drop-out subjects will not be replaced.

Main selection criteria:
Inclusion criteria:

1. *Informed consent*: signed written informed consent before inclusion in the study
2. *Ethnicity, Sex and Age*: Chinese males and females, 18-45 year old inclusive
3. *Weight:* body weight ≥50 kg;
4. *Body Mass Index*: 19-24 kg/m^2 inclusive
5. *Vital signs*: systolic blood pressure 100-139 mmHg, diastolic blood pressure 50-89 mmHg, heart rate 50-90 bpm, measured after 5 min at rest in the sitting/supine position (to be chosen according to the usual procedure at the clinical site)
6. *Full comprehension*: ability to comprehend the full nature and purpose of the study, including possible risks and side effects; ability to co-operate with the investigator and to comply with the requirements of the entire study
7. *Nicotine addiction (smoker subjects only)*: ability to abstain from smoking for the duration of the clinical study
8. *Contraception and fertility (women only)*: women of child-bearing potential must be using at least one of the following reliable methods of contraception:
   a. Hormonal oral, implantable, transdermal, or injectable contraceptives for at least 60 calendar days before the screening visit
   b. A non-hormonal intrauterine device or female condom with spermicide or contraceptive sponge with spermicide or diaphragm with spermicide or cervical cap with spermicide for at least 60 calendar days before the screening visit
   c. A male sexual partner who agrees to use a male condom with spermicide
   d. A sterile sexual partner

   Women of non-child-bearing potential or in post-menopausal status for at least 1 year will be admitted.
   Women of childbearing potential should be willing to adopt abstinence or contraception measures during the study and two weeks post-dose.
   For all women, pregnancy test result must be negative at screening and day -1.

STUDY SYNOPSIS (cont.)

Exclusion criteria:

1. *Electrocardiogram (12-lead ECG in supine position)*: clinically significant abnormalities
2. *Physical findings*: clinically significant abnormal physical findings which could interfere with the objectives of the study
3. *Laboratory analyses*: clinically significant abnormal laboratory values indicative of physical illness, in particular significant laboratory abnormality indicative of hepatic condition (more than 3 times the upper limit)
4. *Allergy*: ascertained or presumptive hypersensitivity to the active principle and/or formulations' ingredients; history of anaphylaxis to drugs or allergic reactions in general, which the investigator considers may affect the outcome of the study
5. *Diseases*: significant history of renal, hepatic, gastrointestinal, cardiovascular, respiratory, skin, haematological, endocrine, urologic, metabolic, neurological or psychiatric diseases, as determined by the investigator, that may interfere with the aim of the study; history of carcinoma *in situ* and malignant disease; active bacterial or viral infection and fever >38° C within 48 h prior to study treatment administration
6. *Virology*: positive result of HIV, hepatitis B (HBV), hepatitis C (HCV) or *Treponema pallidum* (TP) assays
7. *Surgery*: any surgery within 2 months of screening (excluding diagnostic surgery)
8. *Medications*: medications, including over the counter (OTC) medications, herbal remedies and traditional Chinese remedies for 2 weeks before the start of the study. Hormonal contraceptives for women will be allowed
9. *Investigative drug studies*: participation in the evaluation of any investigational product for 30 calendar days before this study
10. *Blood donation*: blood donations for 90 calendar days before this study
11. *Drug, alcohol, caffeine, tobacco*: history of drug, alcohol [>1 drink/day for females and >2 drinks/day for males, defined according to the USDA Dietary Guidelines 2015-2020] caffeine (>5 cups coffee/tea/day) or tobacco abuse (≥10 cigarettes/day)
12. *Abuse drug test*: positive urine abuse drug test at screening or day -1
13. *Alcohol test*: positive alcohol breath test at day -1
14. *Diet*: abnormal diets (<1600 or >3500 kcal/day) or substantial changes in eating habits in the 4 weeks before this study; vegetarians; consumption of alcohol, grapefruit, products containing grapefruit, or beverages containing xanthines (coffee, tea, soda, coffee with milk, energy drinks) within 48 hours prior to the enrolment
15. Pregnancy (females only): positive or missing pregnancy test at screening or day -1, pregnant or lactating women
16. Vaccination within 4 weeks of study treatment
17. Other unspecified reasons that, in the opinion of the investigator, make the subject unsuitable for enrolment.

Schedule:

	Day	Procedures/Assessments	Notes
Screening – Visit 1	*From day -14 to day -2*	➢ Explanation to the subject of study aims, procedures and possible risks ➢ Informed consent signature ➢ Screening number (as [redacted], etc.) ➢ Demographic data and life style recording ➢ Medical/surgical history ➢ Previous/concomitant medications ➢ Full physical examination (body weight, height, vital signs, physical abnormalities) ➢ ECG recording ➢ Clinical laboratory analyses: haematology, blood chemistry, urinalysis, serum virology, bacteriology and pregnancy test (women) ➢ Urine multi-drug kit test ➢ Adverse event (AE) monitoring ➢ Inclusion/exclusion criteria evaluation ➢ Eligibility evaluation	The subject screening numbers will be in running order (e.g. [redacted], etc.). The subject study numbers (those who meet the eligibility at day-1) will be in running order e.g. [redacted], etc. to be identified as the subjects.

STUDY SYNOPSIS (cont.)

Schedule (continued):			
	Day	**Procedures/Assessments**	**Notes**
Visit 2	*Day -1*	➢ Alcohol breath test ➢ Urine multi-drug kit test ➢ Urine pregnancy test (women) ➢ Inclusion/exclusion criteria evaluation ➢ Eligibility evaluation ➢ Enrolment ➢ Subject study number (e.g. CCI, etc.) ➢ Check of AEs and concomitant medications	Arrival at the clinic before the evening (before 18:30) Confinement until the morning of day 8 Standardised dinner Fasting for at least 10 h (overnight) before first dosing
Visit 3	*Day 1*	➢ Vital sign measurement at pre-dose ➢ IMP administration at 08:00 ± 1 h; intravenous infusion for 5 min ➢ Vital signs measurement at 1 h post-dose ➢ Blood sample collection for pharmacokinetic analysis at: pre-dose (0) and 5 (at the end of the infusion), 8, 12, 15, 20, 25, 30, 60 min, 2, 4, 6, 8, 10 and 12 h post-dose ➢ Urine sample collection for pharmacokinetic analysis at: 0-4; 4-8; 8-12 and 12-24 h post-dose ➢ Check of AEs and concomitant medications	Standardized lunch at 13:00 standardized dinner at 21:00
	Day 2	➢ Blood sample collection for pharmacokinetic analysis at 24 and 32 h post-dose ➢ Urine sample collection for pharmacokinetic analysis at 24-32 h post-dose ➢ Check of AEs and concomitant medications	Standardized breakfast around 9:00 Standardized lunch around 13:00 Standardized dinner around 21:00
	Day 3	➢ Vital sign measurement at 48 h post-dose ➢ Physical examination (body weight, physical abnormalities) at 48 h post-dose ➢ Clinical laboratory analyses: haematology, blood chemistry, urinalysis at 48 h post-dose ➢ Check of AEs and concomitant medications	Standardized breakfast around 9:00 Standardized lunch around 13:00 Standardized dinner around 21:00
Visit 4	*From day 4 to day 5*	➢ IMP administration at 08:00 ± 1 h; infusion for 5 min ➢ IMP administration at 20:00 ± 1 h; infusion for 5 min ➢ Blood sample collection for pharmacokinetic analysis at: pre-dose (0) before the morning infusion ➢ Check of AEs and concomitant medications	Standardized breakfast around 9:00 Standardized lunch around 13:00 Standardized dinner around 21:00

STUDY SYNOPSIS (cont.)

Schedule (continued):

	Day	Procedures/Assessments	Notes
Visit 5	*Day 6*	➢ Vital sign measurement at pre-dose ➢ IMP administration at 08:00 ± 1 h; infusion for 5 min ➢ Vital signs measurement at 1 h post-dose ➢ Blood sample collection for pharmacokinetic analysis at: pre-dose (0) and 5 (at the end of the infusion), 8, 12, 15, 20, 25, 30, 60 min, 2, 4, 6, 8, 10 and 12 h post-dose ➢ Urine sample collection for pharmacokinetic analysis at: 0-4; 4-8; 8-12 and 12-24 h post-dose ➢ Check of AEs and concomitant medications	Standardized lunch at 13:00 (about 5 h post-dose) Standardized dinner at 21:00 (about 13 h post-dose)
	Day 7	➢ Blood sample collection for pharmacokinetic analysis at 24 and 32 h post-dose ➢ Urine sample collection for pharmacokinetic analysis at 24-32 h post-dose ➢ Check of AEs and concomitant medications	Standardized breakfast around 9:00 Standardized lunch around 13:00 Standardized dinner around 21:00
Final Visit/ETV	*Day 8 or early termination visit (ETV) in case of discontinuation*	➢ Vital sign measurement at 48 h post-dose (or at ETV) ➢ Physical examination (body weight, physical abnormalities) ➢ Clinical laboratory analyses: haematology, blood chemistry, urinalysis ➢ Check of AEs and concomitant medications In case of clinically significant results at the final visit, the subjects will be followed-up by the investigator until the normalisation of the concerned clinical parameter(s)	Discharge from the clinical centre in the morning of Day 8 after vital sign measurement, blood sampling for clinical laboratory assays and physical examination or in case of ETV. Upon leaving, the subjects will be instructed to contact immediately the investigator in case of occurrence of any untoward medical occurrence

Life style and constraints:
During the study, the subjects will be confined from the evening preceding the first drug administration (study day -1) until the morning of day 8 (after the final visit).
The subjects will not take any food or drinks (except water) for at least 10 h (i.e. overnight) before the first drug administration (Day 1) and will remain fasted up to 5 h post-dose.
During the study, water will be allowed as desired. Coffee, tea or food containing xanthines (i.e. coffee, tea, soda, coffee with milk, energy drinks coke, chocolate, etc.), alcohol and grapefruit will be forbidden starting 48 h prior to the enrolment until the end of the study. Smoking is not allowed for the whole study duration.

STUDY SYNOPSIS (cont.)

Withdrawal of subjects:
It will be documented whether or not each subject completes the clinical study. In case of premature discontinuation of any subject, the primary reason for discontinuation will be recorded.

- Adverse event: Any (significant) adverse event that in the opinion of the investigator or concerned subject is not compatible with study continuation
- death: the absence of life or state of being dead
- lost to follow-up: the loss or lack of continuation of a subject up to follow-up
- non-compliance with study drug: an indication that a subject has not agreed with or followed the instructions related to the study medication
- physician decision: a position, opinion or judgment reached after consideration by a physician with reference to the subject
- pregnancy: pregnancy is the state or condition of having a developing embryo or fetus in the body (uterus), after union of an ovum and spermatozoon, during the period from conception to birth
- protocol deviation: an event or decision that stands in contrast to the guidelines set out by the protocol
- study terminated by sponsor: an indication that a clinical study was stopped by its sponsor
- technical problems: a problem with some technical aspect of a clinical study, usually related to an instrument
- withdrawal by subject: study discontinuation requested by a subject for whatever reason

For any subject discontinuing the study, the investigator will ask the subject to undergo, as far as possible, a final medical visit (ETV) to examine the subject's health conditions and perform the required blood sampling for the laboratory assays. This examination will verify that all values tested at screening have remained within a clinically acceptable range (i.e. not clinically significant changes compared to screening) and report in the case report form (CRF) date and time of the last dose administration, and date and primary reason of study discontinuation.

Data analysis:
The data documented in this trial and the measured clinical parameters will be presented using classic descriptive statistics (i.e. total number of subjects treated [N], number of observations [n], mean standard deviation [SD], minimum [Min], median, maximum [Max]) for quantitative variables and frequencies (i.e. count and percentages) for qualitative variables if not stated otherwise.

Analysis set:
Enrolled set: all enrolled subjects. This analysis set will be used for demographic, baseline and background characteristics
Safety set: all subjects who receive at least one dose of IMP. This analysis set will be used for the safety analyses
PK set: all enrolled subjects who fulfil the study protocol requirements in terms of IMP administration and have evaluable pharmacokinetic data readouts for the planned analysis, with no major deviations that may affect the pharmacokinetic results. This analysis set will be used for the statistical analysis of the pharmacokinetic results

Safety Assessments:
The statistical analysis of demographic and safety data will be performed using the analysis software SAS® .
The toxicity grading of laboratory tests is determined based on the NCI CTCAE V4.03. All AEs, adverse drug reactions and serious adverse events will be summarised by system organ class, severity and relationship to study drug.

Pharmacokinetics:
The statistical analysis of PK parameters will be performed using the validated software Phoenix® WinNonlin® version 6.3 or higher (Certara USA, Inc., Princeton, NJ) (actual version will be stated in the final report).
The individual plasma/urine concentration and pharmacokinetic parameters will be presented in listings and their descriptive statistics summarised in tables.

STUDY SYNOPSIS (cont.)

Background:

N-acetyl-L-cysteine (NAC) is the N-acetyl derivative of the naturally occurring amino acid L-cysteine.

NAC was introduced into the Italian pharmaceutical market in 1965 and it is now manufactured and marketed by Zambon S.p.A. in Europe, Asia, South America, Central America, and the Middle East, under various trade names (Ventresca 1989).

NAC is widely known as a mucolytic agent, with a well-established use in the treatment of respiratory tract disorders. However, after more than 35 years from its introduction in the clinical practice, this molecule continues to be the object of scientific interest due to its potential in the treatment of several conditions other than those related to the respiratory system. NAC can also act as a direct antioxidant agent due to the -SH group (Auroma 1988) and can easily penetrate into the cells where it is deacetylated to L-cysteine, thus supporting the biosynthesis of glutathione (Ziment 1988).

Clinical pharmacology and pharmacokinetics

Several pharmacokinetic (PK) studies were carried out in healthy volunteers (Borgstrom 1986, Olsson 1988, Burgunder 1989, De Caro 1989, Borgstrom 1990, Frascio, Crestani 2002, Rusca 2014, Rusca 2010, Rusca 2002). Further PK studies were carried out in patients with chronic liver damage (Jones 1997), with liver damage caused by paracetamol overdose (Prescott 1989), in subjects under long-term treatment for chemoprevention (Pendyala 1995), in patients with respiratory disorders (Rodenstein 1978) and in patients with End Stage Renal Diseases (Internal report 2002).

In the clinical studies, NAC was administered orally and intravenously both as single and repeated dose. Oral NAC was administered in different dosage forms, such as capsules, granulate, effervescent tablets, fast dissolving tablets, slow-release tablets.

Clinical trials and safety concerns

NAC has been available to patients as a mucolytic agent for many years and has a well-established and satisfactory safety and tolerability profile (see IB).

Independently of route of administration (inhalation, oral, intravenous) adverse events are reported rarely following NAC administration and are usually mild and transient.

The most frequent adverse events associated with NAC oral administration are gastrointestinal (e.g., vomiting, diarrhoea, stomatitis, abdominal pain and nausea). Hypersensitivity reactions (e.g. anaphylactoid reactions, urticaria, rash, pruritus, and bronchospasm) have been reported less frequently after oral administration, while with the IV administration they were reported more frequently compared to gastrointestinal symptoms. Some of the hypersensitivity reactions were associated to higher infusion rate. Most anaphylactoid reactions can be managed by temporarily suspending the acetylcysteine infusion, administering appropriate supportive care. A cumulative analysis of all safety reports received by the Sponsor up to September 2017 for NAC IV, confirmed the nature and severity of the events were in line with the above profile of the product.

Intravenous NAC has been studied in clinical trials and published case series both as mucolytic agent and as an antidote against intoxication by paracetamol and other toxic agents including *Amanita phalloides* and chlorinated hydrocarbons. Overall, in published studies in humans, a total of 745 patients were exposed to i.v. NAC at doses ranging from 500 mg/day to 980mg/kg/day for a duration of 1 to 18 days. Details of safety results are included in the IB.

In published studies, whereas i.v. NAC as mucolytic agent typically was given at doses up to 1000 mg/day, much higher i.v. doses of NAC as antidote were given over multiple days, maintaining a satisfactory safety profile.

Locatelli and colleagues published two case series, one of 86 patients with acute intoxication from *Amanita phalloides* and the other of 35 patients with acute intoxication from chlorinated hydrocarbons. Patients received i.v. NAC 300 mg/kg/day for 3 to 14 days and 3 to 18 days respectively. The safety and tolerability profile were overall benign, with 4 out of 121 exposed patients suffering from anaphylactoid reaction or rash (due to the rapid administration of the bolus according to the authors), none of which caused discontinuation of treatment. No other AEs were reported. (Locatelli 1996).

Rationale

NAC is widely known as a mucolytic agent, with a well-established use in the treatment of respiratory tract disorders. In the present study, the kinetic profile and the bioavailability of NAC will be investigated in Chinese healthy male and female volunteers all receiving the same single i.v. dose, i.e. a total dose of 600 mg of NAC, and the same multiple dose administrations, i.e. five i.v. doses of 600 mg of NAC administered b.i.d. for 2 days and once on the last day. The safety and tolerability will be monitored as well.

The present study will be part of the clinical development plan of NAC i.v. for registration in China of this formulation.

3 STUDY SCHEDULE

ACTIVITIES	Screening	Single Dose				Multiple Dose				Final visit/ETV[1]
Visit	V1	V2	V3			V4		V5		
	Day -14/-2	Day -1	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8[2]
Informed consent	x									
Demography and lifestyle	x									
Medical and surgical history	x									
Physical examination	x				x					x
Prior and concomitant medications	x	x	x	x	x	x	x	x	x	x
Height	x									
Body Weight	x				x					x
Clinical laboratory analysis (haematology, blood chemistry, urinalysis)	x				x					x
Virology and bacteriology	x									
Serum pregnancy test (women)	x									
Urine multi-drug kit test	x	x								
Blood pressure and heart rate	x		x[3]		x			x[3]		x[4]
Alcohol breath test		x								
Urine pregnancy test (women)		x								
ECG	X									
Inclusion/exclusion criteria	X	x								
Subject eligibility	X	x								
Enrolment		x								
Confinement		x	x	x	x	x	x	x	x	
Discharge										x
Investigational product administration			x[5]			x[6]	x[6]	x[5]		
Blood sampling			x[7]	x[7]		x[8]	x[8]	x[7]	x[7]	
Urine sampling			x[9]	x[9]				x[9]	x[9]	
Standardised meals		x[10]	x[11]	x[12]	x[12]	x[12]	x[12]	x[11]	x[12]	
Adverse event monitoring[13]	x	x	x	x	x	x	x	x	x	x

1. Early termination visit (ETV)

2. *Final visit on day 8 or in case of ETV*
3. *At pre-dose and 1 h post-dose*
4. *The vital signs check at 48 h post-dose (day 8), will correspond to the final measurement*
5. *At 8:00 ± 1 h: 5-min infusion*
6. *At 8:00 ± 1 h and at 20:00 ± 1 h; 5-min infusion*
7. *At pre-dose (0) and 5 (at the end of the infusion), 8, 12, 15, 20, 25, 30, 60 min and 2, 4, 6, 8, 10, 12, 24 and 32 h post-dose*
8. *At pre-dose (0)*
9. *0-4; 4-8; 8-12, 12-24 and 24-32 h post-dose*
10. *Standardised dinner*
11. *Standardised lunch and dinner*
12. *Standardised breakfast, lunch and dinner*
13. *Adverse events monitored starting at the screening visit, immediately after informed consent, up to the final visit/ETV*

4 TABLE OF CONTENTS

4.1 TABLES

Page

5 LIST OF ABBREVIATIONS

β-HCG	human chorionic gonadotropin β
γ-GT	γ-Glutamyl transpeptidase
ADR	Adverse Drug Reaction
AE	Adverse Event
ALT	Alanine aminotransferase
Ae_{0-t}	Total amount of NAC excreted in urine
$Aess_{(0-t)}$	Total amount of NAC excreted in urine at steady-state
ANOVA	Analysis of Variance
AST	Aspartate aminotransferase
BB	Blue book
BLQL	Below Lower Quantification Limit
BUN	Blood Urea Nitrogen
$AUC_{(0-t)}$	Area under the concentration-time curve from single dose to the last observed concentration time t
$AUC_{(0-\infty)}$	Area under the concentration-time curve extrapolated to infinity
$AUC_{(0-12)}$	Area under the concentration-time curve at steady-state in the tau interval
$AUCss_{(0-t)}$	Area under the concentration-time curve at steady-state from the last multiple dose to the last observed concentration time t
$AUCss_{(0-12)}$	Area under the concentration-time curve at steady-state in the tau interval
BMI	Body Mass Index
BP	Blood Pressure
CA	Competent Authority
CDISC	Clinical Data Interchange Standards Consortium
CFDA	China Food And Drug Administration
CI	Confidence Interval
C_{max}	Peak drug concentration
CLt	Total body clearance
CLr	Renal clearance
CPL	Clinical Project Leader
CRA	Clinical Research Associate
CRF	Case Report Form
CRO	Contract Research Organisation
CS	Clinically Significant
CSP	Clinical Study Protocol
CSR	Clinical Study Report
C_{ss_av}	Average NAC plasma concentration at steady-state
C_{ss_max}	Maximum plasma concentration at steady-state
C_{ss_min}	Trough plasma concentration at steady-state
CV	Coefficient of Variation
DBP	Diastolic Blood Pressure
DF	Degree of fluctuation
DSU	Drug safety unit
DTT	Dithiothreitol
EC	Ethics Committee
ECG	Electrocardiogram
ETV	Early Termination Visit
FDA	Food and Drug Administration
Fe_{0-t}	Total fraction of NAC dose excreted in urine
F_{rel}	Relative Bioavailability
FSFV	First Subject First Visit
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
HBs Ag	Hepatitis B virus surface antigen
HCV Ab	Hepatitis C virus antibodies
HIV	Human Immunodeficiency Virus

HR	Heart Rate
IB	Investigator's Brochure
ICH	International Conference on Harmonisation
IRB/IEC	Institutional Review Board/Independent Ethics Committee
i.m.	Intramuscular
IMP	Investigational Medicinal Product
IUD	Intra-Uterine Device
i.v.	Intravenous
k_{el}	Terminal elimination rate constant
LQL	Lower Quantification Limit
LSLV	Last Subject Last Visit
MCH	Mean Cell Haemoglobin
MCHC	Mean Cell Haemoglobin Concentration
MCV	Mean Cell Volume
MedDRA	Medical Dictionary for Regulatory Activities
MRT	Mean Residence Time
MW	Molecular Weight
N	Normal
NA	Not Applicable
NAC	N-acetyl-L-cysteine
NC	Not calculated
NCS	Not clinically significant
NSAIDs	Non-Steroidal Anti-Inflammatory Drugs
OTC	Over The Counter
PE	Point Estimate
PK	Pharmacokinetics
PT	Preferred Term
PTAE	Pre-Treatment Adverse Event
R	Accumulation ratio
RBC	Red Blood Cells
RSI	Reference safety information
SAE	Serious Adverse Event
SBP	Systolic Blood Pressure
SD	Standard Deviation
SOC	System Organ Class
SOP	Standard Operating Procedure
SDTM	Study Data Tabulation Model
SUSAR	Suspected Unexpected Serious Adverse Reaction
T	Test
TEAE	Treatment-Emergent Adverse Event
THC	delta-9-tetrahydrocannabinol
$t_{1/2}$	Half-life
t_{max}	Time to achieve C_{max}
t_{ss_max}	Time to achieve C_{ss_max}
USDA	United States Department of Agriculture
Vd	Volume of distribution
WBC	White Blood Cells
WHODDE	World Health Organisation Drug Dictionary Enhanced

6 INTRODUCTION

6.1 Background

N-acetyl-L-cysteine (NAC) is the N-acetyl derivative of the naturally occurring amino acid L-cysteine.

NAC was introduced into the Italian pharmaceutical market in 1965 and it is now manufactured and marketed by Zambon S.p.A. in Europe, Asia, South America, Central America, and the Middle East, under various trade names (1).

NAC is widely known as a mucolytic agent, with a well-established use in the treatment of respiratory tract disorders. However, after more than 35 years from its introduction in the clinical practice, this molecule continues to be the object of scientific interest due to its potential in the treatment of several conditions other than those related to the respiratory system. NAC can also act as a direct antioxidant agent due to the -SH group (2) and can easily penetrate into the cells where it is deacetylated to L-cysteine, thus supporting the biosynthesis of glutathione (3).

6.2 Clinical pharmacology and pharmacokinetics

Several pharmacokinetic (PK) studies were carried out in healthy volunteers (4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14). Further PK studies were carried out in patients with chronic liver damage (15), with liver damage caused by paracetamol overdosage (16), in subjects under long-term treatment for chemoprevention (17), in patients with respiratory disorders (18) and in patients with End Stage Renal Diseases (19).
In the clinical studies, NAC was administered orally and intravenously both as single and repeated doses. Oral NAC was administered in different dosage forms, such as capsules, granulate, effervescent tablets, fast dissolving tablets, slow-release tablets.

In particular, Borgström *et al*. (4) studied for the first time the PK profile of NAC after i.v. dose of 600 mg of NAC infused over 5 min in 10 healthy male and female volunteers in comparison with 3 other oral dosage forms. The PK parameters obtained by the authors after i.v. infusion are summarised in the following table.

Table 6.2.1 PK parameters of plasma free NAC (N=10)

C_{max} (µmol/L)	t_{max} (h)	CL (L/kgxh)	CL_r (L/kgxh)	$t_{½}$ (h)	Ae_{0-12} (% of dose)
16.0±7.9	*0.65±0.33*	*0.207±0.017*	*0.058±0.011*	*2.27±0.32*	*29.0±3.2**

*mean±SD is reported; *: N=9; Source: 4*

However, the authors analysed NAC in deproteinised plasma thus missing the protein-bound NAC in their measurements.

Olsson *et al*. (5) improved the bioanalytical method and were able to measure the total NAC by reduction of the disulphide bonds in plasma before precipitating proteins. After single i.v.

dose of 200 mg of NAC to 6 healthy volunteers, the authors found the following PK parameters:

Table 6.2.2 PK parameters of plasma total NAC (N=6)

	C_{max} (µM)	V_{ss} (L/kg)	CL (L/kgxh)
T	*121 (82.9 – 162)*	*0.47 (0.46 – 0.55)*	*0.11 (0.09 – 1.13)*

median (range) is reported; Source: 5

Jones *et al*. (15) studied the bioavailability of NAC after single i.v. dose of 600 mg of NAC infused over 3 min to patients with chronic liver disease, but also to 6 healthy male and female volunteers as control subjects. The authors found the following PK parameters for total NAC:

Table 6.2.3 PK parameters of plasma total NAC (N=6)

AUC (mg/Lxh)	CL_r (L/h)	$t_{½}$ (h)	Vd_{ss} (L)
93.9±9.6	*6.5±0.8*	*2.6±0.3*	*17.4±2.8*

mean±SD is reported; Source: 15

Brown *et al*. (14) investigated the PK of NAC infused i.v. to 24 healthy men at rest and during exercise. NAC was infused at the dose of 125 mg/kgxh for 15 min followed by 25 mg/kgxh for 35 min to healthy men at rest. Then, the infusion continued during exercise until fatigue. The authors found NAC peak concentrations in plasma at the end of the initial loading infusion. Mean peak concentration of total NAC was 205.1±68.3 mg/L, while mean CL was 0.164 L/kgxh.

It is worth noting that, while scientific literature shows that PK profiles of NAC after oral administration are very consistent (in terms of half-life, rate and extent of exposure, bioavailability, dose-linearity, etc.) among various publications, this is not the case for the data obtained after i.v. dosing. In fact, for this administration route, published studies differ in several key aspects, i.e., for example, type of administration, applied bioanalytical methods, drug doses, calculation methods for PK parameters. Furthermore the number of subjects enrolled in each published study is very small. For these reasons, the comparability of the literature data is low among the published studies and potentially also to the expected results of the present study.

6.3 Clinical trials and safety concerns

NAC has been available to patients as a mucolytic agent for many years and has a well-established and satisfactory safety and tolerability profile (see IB).
Independently of route of administration (inhalation, oral, intravenous) adverse events are reported rarely following NAC administration and are usually mild and transient.
The most frequent adverse events associated with NAC oral administration are gastrointestinal (e.g., vomiting, diarrhoea, stomatitis, abdominal pain and nausea). Hypersensitivity reactions (e.g. anaphylactoid reactions, urticaria, rash, pruritus, and

bronchospasm) have been reported less frequently after oral administration, while with the i.v. administration they were reported more frequently compared to gastrointestinal symptoms. Some of the hypersensitivity reactions were associated to higher infusion rate. Most anaphylactoid reactions can be managed by temporarily suspending the acetylcysteine infusion, administering appropriate supportive care. A cumulative analysis of all safety reports received by the Sponsor up to September 2017 for NAC IV, confirmed the nature and severity of the events were in line with the above profile of the product.
Intravenous NAC has been studied in clinical trials and published case series both as mucolytic agent and as an antidote against intoxication by paracetamol and other toxic agents including *Amanita phalloides* and chlorinated hydrocarbons. Overall, in published studies in humans, a total of 745 patients were exposed to i.v. NAC at doses ranging from 500 mg/day to 980mg/kg/day for a duration of 1 to 18 days. Details of safety results are included in the IB.
In published studies, whereas i.v. NAC as mucolytic agent typically was given at doses up to 1000 mg/day, much higher i.v. doses of NAC as antidote were given over multiple days, maintaining a satisfactory safety profile.
Locatelli and colleagues published two case series, one of 86 patients with acute intoxication from *Amanita phalloides* and the other of 35 patients with acute intoxication from chlorinated hydrocarbons. Patients received i.v. NAC 300 mg/kg/day for 3 to 14 days and 3 to 18 days respectively. The safety and tolerability profile were overall benign, with 4 out of 121 exposed patients suffering from anaphylactoid reaction or rash (due to the rapid administration of the bolus according to the authors), none of which caused discontinuation of treatment. No other AEs were reported (21).

6.4 Rationale

NAC is widely known as a mucolytic agent, with a well-established use in the treatment of respiratory tract disorders.
In the present study, the kinetic profile and the bioavailability of NAC will be investigated in Chinese healthy male and female volunteers all receiving the same single i.v. dose, i.e. a total dose of 600 mg of NAC, and the same multiple dose administrations, i.e. five i.v. doses of 600 mg of NAC administered b.i.d. for 2 days and once on the last day. The safety and tolerability will be monitored as well.
The present study will be part of the clinical development plan of NAC i.v. for registration in China of this formulation.

6.5 Risk and benefits

NAC is a well-known drug which has been used for decades.

Undesired effects which may occur during treatment with NAC include: anaphylactic shock, anaphylactic reaction, anaphylactoid reaction, hypersensitivity, tachycardia, bronchospasm, dyspnoea, vomiting, nausea, angioedema, urticaria, flushing rash, pruritus, face oedema, blood pressure decreased, prothrombin time prolonged (for details refer to IB; 22).

Blood sampling with cannula insertion may cause minor discomfort. The risks associated with blood draws include pain, bleeding and bruising.

No specific benefits for the participants in the current study are foreseen. Their remuneration will be paid after study completion. The remuneration covers loss of time and any inconvenience caused by the participation in the study.

7 STUDY OBJECTIVES

The objective of the study is to evaluate the NAC pharmacokinetics, safety and tolerability after single and multiple dose i.v. administration.

7.1 Primary end-point

- To evaluate pharmacokinetic parameters of NAC in plasma after single and multiple dose administration of the investigational product.

7.2 Secondary end-point

- To collect safety and tolerability data after single and multiple dose administration of the investigational product.

8 CLINICAL SUPPLIES

8.1 Treatment

All the subjects enrolled in the study will receive the same treatment with the investigational medicinal product (IMP), i.e. NAC, 300 mg/ 3 mL solution for injection, as follows:

- on day 1 at 08:00 ±1 h, one dose of 600 mg of NAC (300 + 300 mg ampoule) will be administered under fasting conditions;

After a wash-out of 3 days:

- on days 4 and 5 at 08:00 ±1 h and 20:00 ±1 h and at 08:00 ±1 on day 6, 5 doses of 600 mg of NAC (300 + 300 mg ampoule) will be administered.

8.1.1 Description of products

8.1.1.1 Test product

IMP	NAC 300 mg/ 3 mL solution for injection
Active substance	N-acetyl-L-cysteine
Manufacturer (active substance)	F.I.S. Fabbrica Italiana Sintetici S.p.A., Via Dovaro, 36045 Lonigo (Vicenza), Italy (GMP compliant)
Manufacturer (finished product)	Zambon S.p.A., Via della Chimica 9, 36100 Vicenza, Italy (GMP compliant)
Pharmaceutical form	Solution for injection
Dose	300 mg/ 3 mL
Administration route	Parenteral

The analytical certificates will be supplied with the IMP. Quali-quantitative formulation is as follows:

Each 3 mL vial (10%) contains:
- N-acetyl-L-cysteine (NAC) 300 mg
- sodium hydroxide 74 mg
- disodium edetate 3 mg
- water for injections q.s. to 3 mL

8.1.2 Dose regimen

Two ampoules of IMP (300 + 300 mg) corresponding to a total dose of 600 mg of NAC diluted in 10 mL of NaCl 0.9% sterile saline solution, will be administered by a 5-minute i.v. infusion.

Single dose:

One (1) dose of IMP will be administered under fasting conditions on day 1 at 08:00 ±1 h.

Multiple dose regime:

Five (5) doses of IMP will be administered twice a day (b.i.d.) on days 4 and 5 at 08:00 ±1 h and 20:00 ±1 h and one dose will be administered on day 6 at 08:00 ±1.

8.1.3 Route and method of administration

Each dose of IMP will be prepared by diluting the content of 2 ampoules of IMP in 10 mL of saline, as described above (§ 8.1.2), and administered to each study subject at the clinical site only by the investigator or his/her deputy.

Each dose will be infused intravenously over 5 min.

The start of infusion (not the end) will be considered as time 0.

8.1.4 Investigational product distribution

The IMP will be exclusively used for the present clinical study and will only be administered to the subjects enrolled in the study.

8.2 Packaging and labelling

The IMP primary packaging will be glass vials.

The formulation labelling will report all the information requested according to the Annex 13 to the Good Manufacturing Practice (published by the Commission in The rules governing medicinal products in the European Community, Volume 4; 23) and in compliance with applicable laws and regulations as follows:

a. Name, address and telephone number of the sponsor, contract research organisation or investigator (the main contact for information on the product, clinical study and any emergency)

b. Pharmaceutical dosage form, route of administration, quantity of dosage units, and the name and strength

c. The batch and/or code number to identify the contents and packaging operation

d. A study reference code allowing identification of the study, site, investigator and sponsor if not given elsewhere

e. The study subject identification number/treatment number and where relevant, the visit number

f. The name of the investigator (if not included in (a) or (d))

g. Directions for use (reference may be made to a leaflet or other explanatory document intended for the study subject or person administering the product)

h. "For clinical study use only" or similar wording

i. The storage conditions

j. Period of use (use-by date, expiry date or re-test date as applicable), in month/year format and in a manner that avoids any ambiguity

k. "Keep out of reach of children"

Labels will be in local language.

8.3 Storage conditions

The IMP will be stored at ≤25° C in a dry locked place, sheltered from light.

8.4 Drug accountability

The IMP will be provided directly to the investigator by the Sponsor or designee, in excess of the amount necessary for the study (at least 25% excess).

After receipt of the IMP supply, the pharmacist will confirm in writing by signing and dating standard drug accountability forms.

At the end of the study, used, unused and partially used supplies of IMP provided by the Sponsor or designee will either be destroyed on site (upon written authorisation) or returned to the Sponsor or designee, after assessment of drug accountability.

9 INVESTIGATIONAL PLAN

9.1 Overall study design

This is a single and multiple dose, single centre, open-label, one-way, pharmacokinetics, safety and tolerability clinical trial of Phase I to be performed in Chinese healthy male and female volunteers.

9.2 Discussion of design

The study has been designed in agreement with the Chinese Technical Guideline on Clinical Pharmacokinetic Research of Chemical Drugs, 18 March 2005 and the European Guideline on the Investigation of Bioequivalence (CPMP/QWP/EWP/1401/98 Rev. 1, 20 January 2010) (24).

An open-label design is used since the primary end-point of the study is based on objective measurements of NAC in blood. The outcome variables are not influenced by the subjects or investigator being aware of the administered products.

Blood sampling time-points were selected on the basis of the known PK profile of NAC (4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14).

The dose of 600 mg has been selected since this is the efficacious, safe and well tolerated dose used in clinical practice (see also 22).

The bioanalysis will be performed in compliance with GCP and CFDA GCP regulations and in accordance with the applicable principles of GLP, as defined by OECD, in a GLP compliant facility. Moreover, sample analysis will be conducted in compliance with the Chinese Guidance on Bioanalysis: method validation and analysis of study samples (2015).

10 STUDY POPULATION

10.1 Target population

Chinese healthy male and female volunteers will be included in the study.

10.2 Inclusion criteria

To be enrolled in this study, subjects must fulfil all these criteria:

1. *Informed consent*: signed written informed consent before inclusion in the study
2. *Ethnicity, Sex and Age*: Chinese males and females, 18-45 year old inclusive
3. *Weight:* body weight ≥50 kg;
4. *Body Mass Index*: 19-24 kg/m^2 inclusive
5. *Vital signs*: systolic blood pressure 100-139 mmHg, diastolic blood pressure 50-89 mmHg, heart rate 50-90 bpm, measured after 5 min at rest in the sitting/supine position (to be chosen according to the usual procedure at the clinical site)
6. *Full comprehension*: ability to comprehend the full nature and purpose of the study, including possible risks and side effects; ability to co-operate with the investigator and to comply with the requirements of the entire study
7. *Nicotine addiction (smoker subjects only)*: ability to abstain from smoking for the duration of the clinical study
8. *Contraception and fertility (women only)*: women of child-bearing potential must be using at least one of the following reliable methods of contraception:
    a. Hormonal oral, implantable, transdermal, or injectable contraceptives for at least 60 calendar days before the screening visit
    b. A non-hormonal intrauterine device or female condom with spermicide or contraceptive sponge with spermicide or diaphragm with spermicide or cervical cap with spermicide for at least 60 calendar days before the screening visit
    c. A male sexual partner who agrees to use a male condom with spermicide
    d. A sterile sexual partner

    Women of non-child-bearing potential or in post-menopausal status for at least 1 year will be admitted.

    Women of childbearing potential should be willing to adopt abstinence or contraception measures during the study and two weeks post-dose.

    For all women, pregnancy test result must be negative at screening and day -1.

10.3 Exclusion criteria

Subjects meeting any of these criteria will not be enrolled in the study:

1. *Electrocardiogram (12-lead ECG in supine position)*: clinically significant abnormalities
2. *Physical findings*: clinically significant abnormal physical findings which could interfere with the objectives of the study
3. *Laboratory analyses*: clinically significant abnormal laboratory values indicative of physical illness, in particular significant laboratory abnormality indicative of hepatic condition (more than 3 times the upper limit)
4. *Allergy*: ascertained or presumptive hypersensitivity to the active principle and/or formulations' ingredients; history of anaphylaxis to drugs or allergic reactions in general, which the investigator considers may affect the outcome of the study
5. *Diseases*: significant history of renal, hepatic, gastrointestinal, cardiovascular, respiratory, skin, haematological, endocrine, urologic, metabolic, neurological or psychiatric diseases, as determined by the investigator, that may interfere with the aim of the study; history of carcinoma *in situ* and malignant disease; active bacterial or viral infection and fever >38° C within 48 h prior to study treatment administration
6. *Virology*: positive result of HIV, hepatitis B (HBV), hepatitis C (HCV) or *Treponema pallidum* (TP) assays
7. *Surgery*: any surgery within 60 calendar days of screening (excluding diagnostic surgery)
8. *Medications*: medications, including over the counter (OTC) medications, herbal remedies and traditional Chinese remedies for 2 weeks before the start of the study. Hormonal contraceptives for women will be allowed
9. *Investigative drug studies*: participation in the evaluation of any investigational product for 1 month before this study
10. *Blood donation*: blood donations for 90 calendar days before this study
11. *Drug, alcohol, caffeine, tobacco*: history of drug, alcohol [>1 drink/day for females and >2 drinks/day for males, defined according to the USDA Dietary Guidelines 2015-2020] caffeine (>5 cups coffee/tea/day) or tobacco abuse (≥10 cigarettes/day)
12. *Abuse drug test*: positive urine abuse drug test at screening or day -1
13. *Alcohol test*: positive alcohol breath test at day -1
14. *Diet*: abnormal diets (<1600 or >3500 kcal/day) or substantial changes in eating habits in the 4 weeks before this study; vegetarians; consumption of alcohol, grapefruit, products containing grapefruit, or beverages containing xanthines (coffee, tea, soda, coffee with milk, energy drinks) within 48 hours prior to the enrolment
15. Pregnancy (females only): positive or missing pregnancy test at screening or day -1, pregnant or lactating women
16. Vaccination within 4 weeks of study treatment
17. Other unspecified reasons that, in the opinion of the investigator, make the subject unsuitable for enrolment.

10.3.1 Not allowed treatments

No medication, including OTC, traditional Chinese medicine and herbal remedies, will be allowed for 2 weeks before the start of the study and during the whole study duration. Paracetamol will be allowed as therapeutic counter-measure for adverse events (AEs) according to the investigator's opinion. Hormonal contraceptives will be allowed.

The intake of any other medication will be reported as a protocol deviation.

11 STUDY SCHEDULE

The schedule of the study is summarised at page 11.

11.1 Study visits and procedures

Each study subject will undergo 6 visits.

The study protocol foresees a screening visit and a confinement lasting from Visit 2 to the Final Visit/early termination visit (ETV), i.e. from Day -1 to Day 8. Maximum study duration will be 22 days, screening visit included. A written informed consent will be obtained before any study assessment or procedure.

The first subject first visit (FSFV) is defined as the 1st visit performed at the clinical centre on the 1st screened subject. The last subject last visit (LSLV) is defined as the last visit performed at the clinical centre (or the telephonic follow-up, if applicable) on the last subject, i.e. the last visit foreseen by the study protocol, independently of the fact that the subject is a completer or a withdrawn subject.

The following phases, visits and procedures will be performed:

- **Screening phase**
  - Screening – visit 1: between day -14 and day -2
  - Visit 2: day -1

- **Interventional phase**
  - Visit 3: days 1-3
  - Visit 4: days 4-5
  - Visit 5: days 6-7

- **Final phase**
  - Visit 6: day 8 - Final visit/ETV. In case of early discontinuation, discontinued subjects will undergo an ETV

Activities to be performed are listed by visit in the following table:

Table 11.1.1 Schedule of study activities and procedures

	Day	Procedures/Assessments	Notes
Screening – Visit 1	*From day -14 to day -2*	➢ Explanation to the subject of study aims, procedures and possible risks ➢ Informed consent signature ➢ Screening number (as [redacted], etc.) ➢ Demographic data and life style recording ➢ Medical/surgical history ➢ Previous/concomitant medications ➢ Full physical examination (body weight, height, vital signs, physical abnormalities) ➢ ECG recording ➢ Clinical laboratory analyses: haematology, blood chemistry, urinalysis, serum virology, bacteriology and pregnancy test (women) ➢ Urine multi-drug kit test ➢ AE monitoring ➢ Inclusion/exclusion criteria evaluation ➢ Eligibility evaluation	The subject screening numbers (e.g. [redacted], etc.) and the subject study numbers (e.g. [redacted], etc.) separated by a slash, will be used to identify the subjects
Visit 2	*Day -1*	➢ Alcohol breath test ➢ Urine multi-drug kit test ➢ Urine pregnancy test (women) ➢ Inclusion/exclusion criteria evaluation ➢ Eligibility evaluation ➢ Enrolment ➢ Study subject number (e.g. [redacted], etc.) ➢ Check of AEs and concomitant medications	Arrival at the clinic before the evening (before 18:30) Confinement until the morning of day 8 Standardised dinner Fasting for at least 10 h (overnight) before first dosing
Visit 3	*Day 1*	➢ Vital sign measurement at pre-dose ➢ IMP administration at 08:00 ± 1 h; intravenous infusion for 5 min ➢ Vital signs measurement at 1 h post-dose ➢ Blood sample collection for pharmacokinetic analysis at: pre-dose (0) and 5 (at the end of the infusion), 8, 12, 15, 20, 25, 30, 60 min, 2, 4, 6, 8, 10 and 12 h post-dose ➢ Urine sample collection for pharmacokinetic analysis at: 0-4; 4-8; 8-12 and 12-24 h post-dose ➢ Check of AEs and concomitant medications	Standardized lunch at 13:00 standardized dinner at 21:00
	Day 2	➢ Blood sample collection for pharmacokinetic analysis at 24 and 32 h post-dose ➢ Urine sample collection for pharmacokinetic analysis at 24-32 h post-dose ➢ Check of AEs and concomitant medications	Standardized breakfast around 9:00 Standardized lunch around 13:00 Standardized dinner around 21:00
	Day 3	➢ Vital sign measurement at 48 h post-dose ➢ Physical examination (body weight, physical abnormalities) at 48 h post-dose ➢ Clinical laboratory analyses: haematology, blood chemistry, urinalysis at 48 h post-dose ➢ Check of AEs and concomitant medications	Standardized breakfast around 9:00 Standardized lunch around 13:00 Standardized dinner around 21:00

	Day	Procedures/Assessments	Notes
Visit 4	*From day 4 to day 5*	➢ IMP administration at 08:00 ± 1 h; infusion for 5 min ➢ IMP administration at 20:00 ± 1 h; infusion for 5 min ➢ Blood sample collection for pharmacokinetic analysis at: pre-dose (0) before the morning infusion ➢ Check of AEs and concomitant medications	Standardized breakfast around 9:00 Standardized lunch around 13:00 Standardized dinner around 21:00
Visit 5	*Day 6*	➢ Vital sign measurement at pre-dose ➢ IMP administration at 08:00 ± 1 h; infusion for 5 min ➢ Vital signs measurement at 1 h post-dose ➢ Blood sample collection for pharmacokinetic analysis at: pre-dose (0) and 5 (at the end of the infusion), 8, 12, 15, 20, 25, 30, 60 min, 2, 4, 6, 8, 10 and 12 h post-dose ➢ Urine sample collection for pharmacokinetic analysis at: 0-4; 4-8; 8-12 and 12-24 h post-dose ➢ Check of AEs and concomitant medications	Standardized lunch at 13:00 (about 5 h post-dose) Standardized dinner at 21:00 (about 13 h post-dose)
	Day 7	➢ Blood sample collection for pharmacokinetic analysis at 24 and 32 h post-dose ➢ Urine sample collection for pharmacokinetic analysis at 24-32 h post-dose ➢ Check of AEs and concomitant medications	Standardized breakfast around 9:00 Standardized lunch around 13:00 Standardized dinner around 21:00
Final Visit/ETV	*Day 8 or early termination visit (ETV) in case of discontinuation*	➢ Vital sign measurement at 48 h post-dose (or at ETV) ➢ Physical examination (body weight, physical abnormalities) ➢ Clinical laboratory analyses: haematology, blood chemistry, urinalysis ➢ Check of AEs and concomitant medications In case of clinically significant results at the final visit, the subjects will be followed-up by the investigator until the normalisation of the concerned clinical parameter(s)	Discharge from the clinical centre in the morning of Day 8 after vital sign measurement, blood sampling for clinical laboratory assays and physical examination or in case of ETV. Upon leaving, the subjects will be instructed to contact immediately the investigator in case of occurrence of any untoward medical occurrence

11.2 Diet and lifestyle

The subjects will not take any food or drinks (except water) for at least 10 h (i.e. overnight) before the first drug administration (Day 1) and will remain fasted up to 5 h post-dose.
During the study, water will be allowed as desired. In order to maintain an adequate hydration, the subjects will be encouraged to drink at least 120 mL of mineral water every 2 h for 6 h post-dose on Days 1 and 6.

Coffee, tea or food containing xanthines (i.e. coffee, tea, soda, coffee with milk, energy drinks, etc.), alcohol and grapefruit will be forbidden starting 48 h before the enrolment until the end of the study. Smoking is not allowed for the whole study duration.

During confinement, routine ambulant daily activities will be strongly recommended.

The timing of each meal to be served during confinement is shown in Table11.1.1 above.
In the evening of day -1, upon confinement, the subjects will receive a light dinner before fasting overnight.
On days 1 and 6 (PK sampling collection days), standardised lunch and dinner will be served.
On days 2, 3, 4, 5 and 7, standardised breakfast, lunch and dinner will be served.

11.2.1 Restrictions

During the study, the subjects will be confined from the evening preceding the first drug administration (study day -1) until the morning of day 8 (after the final visit). They will attend the clinic in the evening of day -1 not later than 18:30.

During confinement, hazardous, strenuous or athletic activities will not be permitted.

12 DESCRIPTION OF SPECIFIC PROCEDURES

12.1 Physical examination

Full physical examinations will be performed at the screening visit, visit 3, day 3 and final visit/ ETV. Information about the physical examination will be recorded by the investigator. Any abnormalities will be recorded.

Significant findings/illnesses, reported after the start of the study and that meet the definition of an AE (see § 16), will be recorded in the subject source documents.

Date of the physical examination, overall investigator's interpretation (as normal or abnormal and, if abnormal, clinically significant or not clinically significant) and clinically significant abnormalities (if any) will be reported in the individual CRFs.

12.1.1 Body weight

Body weight will be recorded during each physical examination.
Subjects will be weighed (kg) lightly clothed without shoes. Height will be measured at screening only and BMI will be recorded. BMI will be calculated as weight [kg]/(height [m] x height [m]).

12.1.2 Vital signs

Subjects blood pressure (BP) and heart rate (HR) will be measured by the investigator or his/her deputy after 5 min at rest (in sitting position) at:

- Screening
- Visit 3, Day 1: at pre-dose and 1 h post-dose
- Visit 3, Day 3: at 48 h post-dose
- Visit 5, Day 6: at pre-dose and 1 h post-dose
- Final visit (at 48 h post-dose)/ETV

12.1.3 ECGs

One 12-lead ECG will be performed (in sitting/supine position) at screening only.

12.2 Clinical laboratory assays

Samples of blood and urine will be collected. The following laboratory analyses will be performed at the screening visit:

Haematology
Leukocytes and leukocyte differential count (percentage values and absolute values), erythrocytes, haemoglobin (conv. units), haemoglobin (IS units), haematocrit, MCV, MCH, MCHC, thrombocytes.

Blood chemistry
Electrolytes: sodium, potassium, calcium, chloride, inorganic phosphorus
Enzymes: alkaline phosphatase, γ-GT, AST, ALT
Substrates/metabolites: total bilirubin, creatinine, glucose, BUN or urea, uric acid, total cholesterol, triglycerides
Proteins: total proteins

Serum pregnancy test (women).

Urine analysis
Urine chemical analysis (stick)**:** pH, specific weight, appearance, colour, nitrites, proteins, glucose, urobilinogen, bilirubin, ketones, haematic pigments, leukocytes
Urine sediment (analysis performed only if positive): leukocytes, erythrocytes, flat cells, round cells, crystals, cylinders, mucus, bacteria

Serum virology and bacteriology
Hepatitis B (HBs antigen), **Hepatitis C** (HCV antibodies), **HIV 1/2** (HIV Ag/Ab combo), *Treponema pallidum*.

The same analyses, with the exception of virology, bacteriology and serum pregnancy test, will be performed also at Visit 3, Day 3 (48 h post-dose after the single dose) and at the final visit/ETV.

A urine drug test will be performed at the clinical centre at screening, using a urine multi-drug kit. The following drugs will be assessed: cocaine, amphetamine, methamphetamine, cannabinoids (delta-9-tetrahydrocannabinol - THC), opiates and ecstasy. The same test will be repeated upon confinement at Visit 2, day -1.

A serum pregnancy test will be performed by the laboratory at screening. A urine pregnancy test will be performed at Visit 2, day -1 at the clinical centre, upon confinement.

Date/time of samples collection, overall investigator's interpretation (as normal or abnormal and, if abnormal, clinically significant or not clinically significant) and clinically significant findings (if any) will be reported in the individual CRFs. All clinically significant abnormalities after the screening visit will be recorded as AEs. Hard copies of the laboratory print-outs will be attached to the CRFs.

12.3 Sampling for pharmacokinetic analysis

12.3.1 Venous blood sampling

Venous blood samples (up to 10 mL) will be collected from a forearm vein at the following times, both on Day 1, 2, after the single dose, and on Day 6, 7, after the last multiple dose:

- at pre-dose (0) and 5 (at the end of the infusion), 8, 12, 15, 20, 25, 30, 60 min, 2, 4, 6, 8, 10, 12, 24 and 32 h post-dose

The start of infusion (not the end) will be considered as time 0.

Actual sampling times for each subject will be recorded in the individual case report forms (CRFs). The actual sampling times should not exceed the recommended tolerance ranges presented in the following table. Any deviation outside the recommended ranges will be verified through Data Clarification Forms and, if confirmed, will be reported as protocol deviation, although it will not automatically lead to the exclusion of the concerned subjects from the PK analysis set.

Table 12.3.1.1 Tolerance ranges for the scheduled sampling times

Sampling time	Tolerance range
Pre-dose (0)	Within 30 minutes before IMP administration
0.0833 h (5 min), 0.1333 (8 min) 0.200 h (12 min), 0.250 h (15 min)	20 seconds
0.3333 h (20 min), 0.4167 (25 min), 0.5 h (30 min)	± 1 min
1 h	± 3 min
2, 4 h	± 5 min
6, 8, 10, 12, 24, 32 h	± 10 min

Blood samples for PK analysis will be collected using an indwelling catheter with switch valve. The cannula will be rinsed, after each sampling, with about 1 mL of sterile saline solution. The first mL of blood will be discarded at each collection time.

The remaining blood sample will be collected into heparinised tubes (Li-heparin).

Storage on ice will not last longer than 10 min until centrifugation at 4° C for 10 min at 2500xg to obtain plasma. Plasma will be collected into separate tubes, where an appropriate volume of DTT solution (50 µL DTT solution/mL plasma, i.e. 5% of the plasma volume according to the ratio 75 µL of DTT solution for 1.5 mL of plasma) will be added. The samples will be mixed shortly (Vortex, 20 sec), divided into two aliquots, P1 and P2 and transferred to ≤-70° C as soon as possible. The DTT solution (5 mg DTT/mL of water) will be freshly prepared in the evening before each sampling day and kept refrigerated until use. The preparation of the solution will be recorded in appropriate forms.

If any clinical assessment, such as vital signs measurement, is foreseen at the same time-point as blood sampling for PK analysis, blood collection will be performed at the scheduled time. However, vital signs can be influenced by the blood sampling. Therefore, these assessments can be performed within 30 min before the pre-dose PK time point (0 h) and within 10 min before the other scheduled PK time-points. Any deviations outside the recommended time will be verified through Data Clarification Forms. However, since vital signs measurements will be performed for safety reasons only, deviations from the planned time schedule will be considered not relevant.

12.3.2 Urine collection

Urine for PK will be collected in the following time intervals both on Day 1, 2, after the single dose, and on Day 6, 7 after the last multiple dose:

- 0-4; 4-8; 8-12, 12-24 and 24-32 h post-dose

Bladder must be emptied before the end of each collection period. During each interval, urine will be collected into containers, kept refrigerated at approximately 4° C and containing DTT solution. At the end of each collection interval, urine volume will be measured and after thorough mixing, two aliquots of 10 mL each (U1 and U2) will be prepared in polypropylene tubes. Urine volume collected in each collection interval will be accurately recorded in appropriate forms and in the CRFs. The two aliquots will be stored at ≤-70° C. The DTT solution (5 mg DTT/mL of water) will be freshly prepared in the evening before each sampling day and kept refrigerated until use. The preparation of the solution will be recorded in appropriate forms.

12.3.3 Analytics

The concentration of total NAC in plasma and urine will be determined at a certified bioanalytical laboratory to be designated in China, using a fully validated LC-MS/MS method, with a lower quantification limit (LQL) of 10 ng/mL.

Analyses will be performed according to the general Principles of "OECD Good Laboratory Practices for testing of chemicals" C(81) 30 (final) and GCP/ CFDA GCP (see also § 9.2).

The method validation report and the analytical report will be attached to the final report.

12.3.4 Labelling, storage and transport of samples

12.3.4.1 Samples labelling

Each sample tube will be clearly and unequivocally identified with a label resistant to the storage temperature and reporting:

Study code	Sponsor code Z7244J01
Subject number	Subject or Process or Unique Subject Identification number, e.g. CCI etc.
Tube identification	CCI
Study day	1 or 6
Scheduled sampling time	as min and h; see § 12.3.1

12.3.4.2 Samples storage and transport

During the study the samples will be stored at ≤-70° C. At the end of each collection day, aliquots 1 and 2 will be stored in separate freezers.

All aliquots 1, packed in sufficient solid CO_2, will be shipped by an authorised courier from the clinical site, China, to Eurofins Central Laboratory, Shanghai, China. Aliquots 1 will remain stored at Eurofins Central Laboratory or a subcontracted bioanalytical laboratory to be designated until finalisation of the bioanalytical report. Afterwards, the samples will be destroyed and a certificate of destruction will be provided to the sponsor.

The counter-samples (aliquot 2) will remain stored at the clinical site, China. These samples could either be:

- sent to the laboratory for reanalysis should this become necessary for analytical reasons or if any problems occur during the delivery of aliquots 1, or
- destroyed at an authorised site, or
- transferred to the sponsor upon written request.

No analyses different from those stated in this protocol and agreed by the subjects when signing the informed consent form will be performed unless a new informed consent and a new approval from the Ethical Committee is obtained. The subjects may ask to destroy their own samples at any time.

13 ASSIGNMENT OF STUDY TREATMENT

13.1 Randomisation

No randomisation will take place in the present study. All the subjects will receive the same treatment.

13.2 Blinding

This is an open study. No masking procedure will be applied.

14 EVALUATION PARAMETERS

14.1 Study variables

14.1.1 Primary variables

After single dose:

- C_{max}, t_{max}, k_{el}, $t_{1/2}$, $AUC_{(0-t)}$, $AUC_{(0-\infty)}$, $AUC_{(0-12)}$, V_d, CL_t, $Ae_{(0-t)}$, $Fe_{(0-t)}$ and CL_r

After multiple doses:

- C_{ss_max}, t_{ss_max}, C_{ss_min}, $AUCss_{(0-t)}$, $AUCss_{(0-12)}$, C_{ss_av}, R, DF% and $Aess_{(0-t)}$.

14.1.2 Secondary variables

- TEAEs, vital signs (BP, HR), body weight, physical examinations, laboratory parameters.

14.2 Pharmacokinetic assessments

14.2.1 Pharmacokinetic parameters

The following PK parameters will be measured and/or calculated for plasma NAC, using the validated software Phoenix® WinNonlin® version 6.3 or higher (Certara USA, Inc., Princeton, NJ) (actual version will be stated in the final report), after single dose of IMP:

C_{max}:	Maximum NAC plasma concentration
t_{max}:	Time to achieve C_{max}
k_{el}:	Terminal elimination rate constant, calculated, if feasible, by log-linear regression using at least 3 points
$t_{1/2}$:	Half-life, calculated, if feasible, as $ln2/k_{el}$
$AUC_{(0-t)}$:	Area under the concentration-time curve from single dose to the last observed concentration time t, calculated with the linear up/log down trapezoidal method
$AUC_{(0-\infty)}$:	Area under the concentration-time curve extrapolated to infinity, calculated, if feasible, as $AUC_{(0-t)} + C_t/k_{el}$, where C_t is the last measurable drug concentration
$AUC_{(0-12)}$:	Area under the concentration-time curve at steady-state in the tau interval (from single dose to 12 h post-dose), calculated with the linear up/log down trapezoidal method

V_d: Volume of distribution associated with the terminal slope, calculated, if feasible, as Dose/($AUC_{(0-\infty)}$*k_{el})

CL_t: Total body clearance, calculated, if feasible, as Dose/ $AUC_{(0-\infty)}$

$Ae_{(0-t)}$: Total amount of NAC excreted in urine from single dose up to 32 h

$Fe_{(0-t)}$: Total fraction of NAC dose excreted in urine from single dose up to 32 h

CL_r: Renal clearance, calculated, if feasible, as $Ae_{(0-t)}$/ $AUC_{(0-\infty)}$

The following PK parameters will be measured and/or calculated for plasma NAC, using the same software, after multiple doses of IMP:

C_{ss_max}: Maximum NAC plasma concentration at steady-state

t_{ss_max}: Time to achieve C_{ss_max}

C_{ss_min}: Trough NAC plasma concentration at steady-state, measured as concentration at t=12 h

$AUCss_{(0-t)}$: Area under the concentration-time curve at steady-state from the last multiple dose to the last observed concentration time t, calculated with the linear up/log down trapezoidal method

$AUCss_{(0-12)}$: Area under the concentration-time curve at steady-state in the tau interval (from the last multiple dose to 12 h post-dose), calculated with the linear up/log down trapezoidal method

C_{ss_av}: Average NAC plasma concentration at steady-state, calculated as $AUCss_{(0-12)}$ /tau

R: Accumulation ratio, calculated as $AUCss_{(0-12)}$/ $AUC_{(0-12)}$

$Aess_{(0-t)}$: Total amount of NAC excreted in urine from the last multiple dose to 32 h at steady-state

DF%: Degree of fluctuation over one dosing interval at steady-state, calculated as (C_{ss_max} - C_{ss_min})/ C_{ss_av}*100

The sampling schedule is considered adequate if the ratio $AUC_{(0-t)}$/ $AUC_{(0-\infty)}$ equals or exceeds a factor of 0.8 (i.e. if $\%AUC_{extra}$ is <20%) for more than 80% of the individual PK profiles. This assures that the primary variable $AUC_{(0-t)}$ covers a sufficient percentage of the theoretical total extent of exposure.

The quality of log-linear regression (and, consequently, the reliability of the extrapolated PK parameters) should be demonstrated by a determination coefficient $R^2 \geq 0.8$. Individual extrapolated parameters, when considered unreliable, will be reported as NC (not calculated).

14.3 Safety assessments

Safety and general tolerability of the IMP will be based on TEAEs, physical examinations including body weight, vital signs and routine haematology, blood chemistry and urinalysis laboratory tests.

15 STATISTICAL METHODS

The data documented in this trial and the measured clinical parameters will be presented using classic descriptive statistics (i.e. total number of subjects treated [N], number of observations [n], mean standard deviation [SD], minimum [Min], median, maximum [Max]) for quantitative variables and frequencies (i.e. count and percentages) for qualitative variables if not stated otherwise.

Not available data will be evaluated as "missing values". The statistical analysis of demographic and safety data will be performed using the analysis software SAS® .

The statistical analysis of PK parameters will be performed using the validated software Phoenix® WinNonlin® version 6.3 or higher (Certara USA, Inc., Princeton, NJ) (actual version will be stated in the final report).

15.1 Analysis Sets

15.1.1 Definitions

A subject will be defined as screened after the signature of the informed consent, regardless of the completion of all the screening procedures.

A subject will be defined as eligible if he/she meets all the inclusion/exclusion criteria. Otherwise he/she will be defined as a screen failure.

A subject will be defined as enrolled in the study if he/she is included into the interventional phase of the study.

The following analysis sets are defined:

- Enrolled set: all enrolled subjects. This analysis set will be used for demographic, baseline and background characteristics
- Safety set: all subjects who receive at least one dose of IMP. This analysis set will be used for the safety analyses
- PK set: all enrolled subjects who fulfil the study protocol requirements in terms of IMP intake and have evaluable PK data readouts for the planned analyses, with no major deviations that may affect the PK results. This analysis set will be used for the statistical analysis of the PK parameters.

Each subject will be coded as valid or not valid for the safety and the PK set. Subjects will be evaluated according to the treatment they actually receive.

15.1.2 Reasons for exclusion from the PK set before bioanalysis

Reasons for the exclusion of subjects from the PK set are the following:

- intake of concomitant medications which could render the plasma concentration-time profile unreliable
- AEs which could render the plasma concentration-time profile unreliable
- administration errors which could render the plasma concentration-time profile unreliable
- other events which could render the plasma concentration-time profile unreliable

If one of these events occurs, it will be noted in the CRF during the study.

The samples from the subjects excluded from the PK set should still be assayed and the results listed. Subjects should not be excluded from the PK set if the AUC_{0-t} covers less than 80% of the $AUC_{0-\infty}$.

15.2 Sample size and power considerations

The sample size was not calculated through any statistical calculation. A sample size of 24 subjects was estimated as sufficient for the descriptive purposes of the present study.
Drop-out subjects will not be replaced.

15.3 Demographic, baseline and background characteristics

Critical demographic characteristics will be examined according to qualitative or quantitative data. Qualitative data will be summarised in contingency tables. Quantitative data will be summarised using classic descriptive statistics.

15.4 Analysis of pharmacokinetic parameters

15.4.1 Descriptive pharmacokinetics

A descriptive PK will be presented. The results will be displayed and summarised in tables and figures. Individual and mean curves (+SD at sampling times), indicating inter-subject variability, will be plotted. Data below the lower quantification limit (BLQL) will be considered as 0 in the calculations and presented as BLQL in listings and tables. As a consequence of BLQL (i.e. 0) values, calculated geometric means (if requested) could be null. For this reason, in the presence of any null value, the geometric mean will be reported as not calculated (NC).

15.5 Safety and tolerability evaluation

- **AEs**

AEs will be coded by System Organ Class (SOC) and Preferred Term (PT), using the Medical Dictionary for Regulatory Activities (MedDRA).
AEs will be classified as pre-treatment AEs (PTAEs) and TEAEs, according to the period of occurrence, as follows:

- PTAEs: all AEs occurring before the first dose of IMP and not worsening after the first dose of IMP
- TEAEs: all AEs occurring or worsening after the first dose of IMP

Individual PTAEs and TEAEs will be listed in subject data listings. No summary table will be provided for PTAEs. TEAEs will be summarised by treatment and overall. The number and percentage of subjects with any TEAE and the number of TEAEs will be tabulated by SOC and PT, seriousness, relationship to treatment and severity.

- **Physical examination**

Date of the physical examination, overall investigator's interpretation (as normal or abnormal and, if abnormal, clinically significant or not clinically significant) and clinically significant abnormalities (if any) will be listed.

- **Laboratory data**

Date/time of samples collection, overall investigator's interpretation (as normal or abnormal and, if abnormal, clinically significant or not clinically significant) and clinically significant findings (if any) will be listed. All laboratory results will be listed and a table of all the abnormal values will be presented. The overall investigator's interpretation will be summarised using tables of frequency.
The toxicity grading of laboratory tests is determined based on the NCI CTCAE V4.03.

- **Vital signs**

Vital signs values will be listed and summarised by descriptive statistics.

- **Body weight**

Body weight values will be listed and summarised by descriptive statistics.

16 DEFINITION AND HANDLING OF AEs AND SAEs

16.1 Applicable SOPs

AEs definition, classification and management will follow the CRO's SOPs, based upon applicable local and international regulations. The full SOPs or an operative summary will be made available to the clinical centre.

A brief summary of AE definition, classification and management is reported below.

16.2 Definition of Adverse Event (AE)

An Adverse Event is "*any untoward medical occurrence in a patient or clinical trial subject administered a medicinal product and which does not necessarily have a causal relationship with this treatment*".

AEs include:

- worsening (change in nature, severity or frequency) of conditions present at the onset of the trial
- patient/subject deterioration due to the primary illness
- intercurrent illnesses
- drug interactions
- events related or possibly related to concomitant medications
- abnormal laboratory values, as well as significant shifts from baseline within the range of normal, which the investigator considers to be clinically significant.

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

16.3 Definition of Adverse Drug Reaction (ADR)

An Adverse Reaction is "*any untoward and unintended response to an investigational medicinal product related to any dose administered*".
All AEs judged by either the reporting investigator or the sponsor as having a reasonable causal relationship to a medicinal product qualify as adverse reactions. The expression reasonable causal relationship means to convey in general that there is evidence or matter to suggest a causal relationship.

- **Unexpected Adverse Drug Reaction**

An unexpected ADR is: "*An adverse reaction, the nature, or severity of which is not consistent with the applicable product information (Reference Safety Information [RSI]).*

- **Reference Safety Information (RSI)**: in order to assess whether an adverse reaction is expected, the IB will be used.

16.4 Definition of Serious Adverse Events or Serious Adverse Drug Reaction

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 (i.e. the subject was at risk of death at the time of the event/reaction; it does not refer to an event/reaction which hypothetically might have caused death if it were more severe)
- requires inpatient hospitalization or prolongation of existing hospitalization
- results in persistent or significant disability/incapacity (where disability is defined as a permanent or substantial disruption of ability to carry out normal life functions, either reported or defined as per clinical judgement)
- is a congenital anomaly/birth defect
- is an important medical event that may not result in death, be life-threatening, or require hospitalization but, according to appropriate medical judgment, it may jeopardize the subject and may require medical or surgical intervention to prevent any of the outcomes listed in the definition above. Examples of such events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, and blood dyscrasias or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse.

Any suspected transmission via a medicinal product of an infectious agent is also considered a serious adverse event.

A **Suspected Unexpected Serious Adverse Reaction** (SUSAR) is an ADR that is both unexpected (not consistent with the applicable product information, e.g. IB) and also meets the definition of a SAE.

A non-serious adverse event is any adverse event that does not meet the criteria listed above for a serious adverse event.

16.5 Definition of Severity of Adverse Events

The term "severe" is used to describe the intensity (severity) of a specific event:

- Mild: causing no limitation of usual activities; the subject may experience slight discomfort.
- Moderate: causing some limitation of usual activities; the subject may experience annoying discomfort.
- Severe: causing inability to carry out usual activities; the subject may experience intolerable discomfort or pain.

16.6 Definition of Adverse Event causality

Causality shall be determined according to the definition of ADR given in 16.3.

All AE judged by either the investigator or the sponsor as having a reasonable suspected causal relationship to an investigational medicinal product qualify as adverse reactions. The causality assessment given by the investigator should not be downgraded by the sponsor.

The following binary decision for causality will be used:

- Reasonable possibility that the IMP caused the event
- No reasonable possibility that the IMP caused the event

Features supportive of an association include:

- temporal plausibility
- pharmacological properties of the drug or of the substance class
- course of the adverse event after dechallenge and, if applicable, after rechallenge
- specific tests indicating involvement of the drug in the occurrence/worsening of the adverse event
- alternative explanations

16.7 Adverse Events recording

Each AE occurring to a subject, either spontaneously revealed by the subject or observed by the investigator, whether believed by the investigator to be related or unrelated to the IMP, must be recorded on the AE information page of the CRF (for SAEs information must be recorded also on the "Serious Adverse Event Form").
The Investigator will also perform an evaluation with respect to seriousness and causality (relationship of any AE to IMP) of the AEs. and record it on the appropriate section of the CRF and on the "Serious Adverse Event Form" (if appropriate).

16.8 AEs monitoring window

- Start of monitoring: from immediately after the signature of the informed consent
- End of monitoring: final study visit/ETV (observation window)

An AE occurring after the final visit/ETV and coming to knowledge of the investigator (e.g. during a follow-up assessment or by spontaneous reporting by study subjects) must be recorded only if it is an ADR, according to the investigator's judgment.

16.9 Adverse Events reporting

The official language for reporting is English. The investigator and clinical staff of the present study are familiar with English language.

The investigator must report to the CRO all AEs which occur during the study, regardless of their relationship to the IMP. Protocol specific AEs or laboratory abnormalities critical to safety evaluations are to be identified in the protocol and reported to the sponsor according to reporting requirements and within the time periods specified.

All AEs are recorded by the investigator on the AE information page of the CRF.

In addition, SAE will have to be reported according to the following detailed procedure.

16.9.1 SAEs reporting

With the exception of those SAEs that are identified as not requiring immediate reporting in the protocol, the investigator must report the SAEs to the CRO immediately and no later than 24 hours from when he/she becomes aware of the SAE, by faxing the "Serious Adverse Event Form" (back up plan) or e-mailing as scanned attachment (backup plan) or by Electronic Data Capture to the Drug Safety Unit (DSU) personnel (preferred method), as stated in the "List of CRO/ personnel" in § 16.13 of this protocol.

The community standards of confidentiality must always be maintained and any relevant national legislation on data protection must be followed.

Another copy of the "Serious Adverse Event Form" will be retained by the Investigator for the Investigator's file.

If the investigator becomes aware of any SAE occurred to a subject within the follow-up window established in the protocol, he/she will report the SAE as above. The SAE will be also reported in the CRF.

If outside the follow-up window established in the protocol the investigator becomes aware of a SAE, it is the investigator's responsibility to report the SAE to the CRO. The Investigator might use the "Serious Adverse Event Form" via email or fax, but the SAE must not to be reported in the CRF, as it is not an event occurred within the study period.

16.10 Follow-up for Adverse Events

A follow-up "Serious Adverse Event Form" will be filled in if important follow-up information (i.e. diagnosis, outcome, causality assessment, results of specific investigations) is made available after submission of the initial AE Form for immediate reporting. Follow-up "Serious Adverse Event Form" will be reported to the Sponsor as above-described, under Section 16.9.1.

In any case of an AE that, in the opinion of the investigator, requires the subject's discontinuation, follow-up information relating to the subjects subsequent course must be collected until the event has subsided or the condition stabilised or when the subject is lost to follow up and uncontactable.

When follow-up data on non-serious AE are collected, information should be reported under "Comments" in the Final report of the CRF.

16.11 SUSARs management

The clock for initial expedited reporting starts as soon as the information containing the minimum reporting criteria has been received by the sponsor (day 0).
For fatal and life-threatening SUSARs the EC and Competent Authority (CA) should be informed as soon as possible and in any case within 7 days.
If the initial report is incomplete, e.g. not all the information/assessments were available, a complete report should be sent within an additional 8 days. Complete follow-up report should be sent within 15 calendar days.
SUSARs which are not fatal and not life-threatening are to be reported within 15 days.
The minimum information to be reported includes:

- Sponsor study number
- One identifiable coded subject
- One identifiable reporter
- One SUSAR
- One suspect IMP (including active substance name, code)
- A causality assessment (a reasonable possibility of a causal relationship with the study drug can be excluded only if there is information supporting this decision, otherwise it cannot be excluded).

In addition, in order to properly process the report electronically, the following administrative information should be provided:

- the sender's (case) safety report unique identifier,
- the receipt date of the initial information from the primary source,
- the receipt date of the most recent information,
- the worldwide unique case identification number,
- the sender identifier.

16.12 Other events qualified for expedited reporting

Other safety issues also qualify for expedited reporting when they might materially alter the current benefit-risk assessment of a medicinal product or would be sufficient to consider changes in the medicinal product administration or in the overall conduct of the trial, for instance:

- single case reports of an expected serious adverse reaction with an unexpected outcome (e.g. a fatal outcome)
- an increase in the rate of occurrence of an expected serious adverse reaction, which is judged to be clinically important.
- post-study SUSARs that occur after the subject has completed a clinical trial and are reported to the investigator by the subject.
- new events relating to the conduct of the trial or the development of the medicinal product likely to affect the safety of the subjects, such as :
  - a SAE which could be associated with the trial procedures and which could modify the conduct of the trial

- a significant hazard to the subject population such as lack of efficacy of a medicinal product used for the treatment of a life-threatening disease
- a major safety finding from a newly completed animal study (such as carcinogenicity) or from other clinical trials.

16.13 SAEs: contacts

The investigator will report any SAE to the CRO. The CRO's details for SAEs are the following:

CCI

CCI

16.14 Pregnancy

Subjects must be instructed that known or suspected pregnancy occurring during the study should be confirmed and reported to the Investigator, who must then withdraw the subject from the study without delay. The Investigator should also be notified of pregnancy occurring during the study but confirmed after its completion. In the event that a subject is subsequently found to be pregnant after inclusion in the study, then any pregnancy will be followed to term and the status of mother and child will be reported to the Sponsor after delivery through the Pregnancy Report Form provided by the Sponsor. The Investigator will send pregnancy reports to DSU within the timeframes of SAEs, with follow-up information to be actively sought for the outcome of pregnancy.

- Part I of the Form is completed at the time of awareness of foetal exposure during pregnancy.
- Part II of the Form is filled in when information on pregnancy outcome becomes available.

If pregnancy results in abnormal outcome that the investigator considers to be due to the IMP, this will be treated as an expedited ADR report.

17 DATA MANAGEMENT PROCEDURES

17.1 Data collection – CRFs

The investigator must ensure that the clinical data required by the study protocol are carefully reported in the CRFs. He/she must also check that the data reported in the CRFs correspond to those in the subject's source documents.
To ensure legibility, the CRFs should be filled out in English, in block capitals with a ball-point pen (not pencil, felt tip or fountain pen). Any correction to the CRFs' entries must be carried out by the investigator or a designated member of staff. Incorrect entries must not be covered with correcting fluid, or obliterated, or made illegible in any way. A single stroke must be drawn through the original entry. Corrections have to be dated and initialled. In the interest of completeness of data acquisition, the questions which are repeated in each section of the CRFs should be answered in full, even if there are no changes from a previous examination. The investigator must provide a reasonable explanation for all missing data.
The CRFs will be completed, signed by the investigator, sent to the CRO for data management procedures and finally sent to the sponsor.

17.2 Database management

The CRO will provide a data entry and will update and verify the database and create the final data sets. The final data file will be transferred to the sponsor in the agreed format with all the other study documentation.

17.2.1 Coding dictionaries

Medical/surgical history and underlying diseases, clinically significant physical examination abnormalities and AEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA™).
Previous and concomitant medications will be coded using the WHO Drug Dictionary Enhanced (WHODDE). The version of the coding dictionaries will be stated in the study report.

18 STUDY MONITORING, QUALITY CONTROL AND QUALITY ASSURANCE

18.1 Monitoring

The monitoring visits will be conducted by appropriate staff of PAREXEL China Co. Ltd according to PAREXEL SOPs.

Monitoring activities, including monitoring purpose, selection and qualifications of monitors, extent and nature of monitoring, monitoring procedures, monitoring reports will comply with ICH-GCP chapter 5.18, CFDA GCP requirements and, when appropriate, further national regulatory guidelines.

Adequate time and availability for monitoring activities should be ensured by the investigator and key study personnel.

Data verification is required and will be done by direct comparison with source documents, always giving due consideration to data protection and medical confidentiality. In this respect the investigator will assure support to the monitor at all times.

The investigator agrees, by written consent to this protocol, to fully co-operate with compliance checks by allowing authorised individuals to have access to all the study documentation. In addition to the monitoring activities performed by the study monitor, the sponsor could perform some quality control activities to verify the compliance with the study procedures, the ICH-GCP guidelines and CFDA GCP.

18.2 Quality Control and Quality Assurance

The CRO has implemented and maintains a Quality System that includes quality controls and audits at different study steps with written SOPs to ensure that the study is conducted in compliance with the protocol and all effective amendments, ICH-GCP, and the applicable regulatory requirement(s) and that data have been reliably and correctly generated, recorded, processed and reported, in agreement with the ALCOAC principles (Attributable-Legible-Contemporaneous-Original-Accurate-Complete).

The clinical site is responsible for implementing and maintaining quality assurance and a quality control system to ensure that the study is conducted, and data are generated, documented (recorded), and reported in compliance with the protocol, ICH-GCP, CFDA GCP and any applicable regulatory requirement(s).
This protocol has been audited by the Sponsor QA.
The CRO and the sponsor will be responsible for their respective activities.

The sponsor may transfer any or all of the sponsor's trial-related duties and functions to a CRO, but the ultimate responsibility for the quality and integrity of the trial data always resides with the sponsor.

18.3 Applicable SOPs

The sponsor, the clinical centre and the CRO will follow their respective SOPs in the conduct of the respective activities, unless otherwise stated in written agreements. CRO/Sponsor's SOPs will be used for AE and SAE definition and management. SOPs will be made available for Sponsor’s review, if required.

18.4 Data access

The investigator and the CRO will ensure that all source data, medical records, CRFs and all other documentation that is relevant to this study will be made accessible for monitoring activities, audits, IEC review, and regulatory inspections.

18.5 Audits and inspections

The sponsor, independent bodies acting on behalf of the sponsor and the CRO have the right to perform audits according to ICH-GCP, CFDA GCP and CFDA responsibilities.

The study may also be inspected by regulatory authorities.

The investigator and the CRO agree, by written consent to this protocol, to fully co-operate and support audits and inspections compliance checks by allowing authorised individuals to have access to all the study documentation.

19 ETHICAL CONSIDERATIONS

19.1 Ethics and Good Clinical Practice (GCP)

The study will be performed in accordance with the relevant guidelines of the Declaration of Helsinki.
The approval of the study protocol by the local IEC and by the Chinese Health Authorities will be obtained before the start of the study.

The present clinical study will be carried out according to the current revision of Good Clinical Practice (GCP), ICH topic E6 (R2), CFDA GCP and any applicable local law requirements.

19.2 Informed consent

Before being enrolled into the clinical study, the subjects must have expressed their consent to participate, after the investigator has explained to them, clearly and in details, the scope, the procedures and the possible consequences of the clinical study. Information will be given in both oral and written form. The information sheet and informed consent form will be prepared in the local language by the CRO and must be approved by the EC and regulatory authorities. It will include all the elements required by law according to the ICH-GCP and CFDA GCP recommendations.
In addition to the standard requirements that physicians are currently obliged to observe when providing information, the following points must also be covered:

- a description of the aims of the study and how it will be organised
- the type of treatment (information on the IMP and treatment procedures, as applicable)
- any potential negative effects attributable to the study product or treatment
- the freedom to ask for further information at any time
- the subjects' right to withdraw from the clinical study at any time without giving reasons and without jeopardising their further course of medical treatment
- the existence of a subject insurance cover and obligations following from this cover

Adequate time and opportunity to satisfy questions will be given to the subjects and the time will be recorded.

The investigator will be supplied with an adequate number of blank informed consent forms to be used. The forms will be signed and dated by both the investigator and the subject.
A copy of the signed form will be given to the subject.

To ensure medical confidentiality and data protection, the signed informed consent forms will be stored in the investigator's study file according to the regulatory requirements (see § 20.3). The investigator will allow inspection of the forms by authorised representatives of the sponsor, EC members and regulatory authorities. He/she will confirm, by signing and dating the forms, that informed consent has been obtained.

19.3 Insurance policy

An insurance cover has been issued in favour of the subjects participating in this clinical study. The insurance is in compliance with the local regulation and with the requirements of the Health Authorities.

19.4 Withdrawal of subjects

It will be documented whether or not each subject completed the clinical study. If, for a subject, study treatment or observations are discontinued, the primary reason for discontinuation will be recorded.

19.4.1 Primary reason for discontinuation

- **Adverse event**: Any (significant) adverse event that in the opinion of the investigator or concerned subject is not compatible with study continuation. For the definition of AE, please refer to § 16.2.
- **death**: the absence of life or state of being dead
- **lost to follow-up**: the loss or lack of continuation of a subject to follow-up
- **non-compliance with study drug**: an indication that a subject has not agreed with or followed the instructions related to the study medication
- **physician decision**: a position, opinion or judgment reached after consideration by a physician with reference to the subject
- **pregnancy**: pregnancy is the state or condition of having a developing embryo or fetus in the body (uterus), after union of an ovum and spermatozoon, during the period from conception to birth
- **protocol deviation**: an event or decision that stands in contrast to the guidelines set out by the protocol
- **study terminated by sponsor**: an indication that a clinical study was stopped by its sponsor
- **technical problems**: a problem with some technical aspect of a clinical study, usually related to an instrument
- **withdrawal by subject**: study discontinuation requested by a subject for whatever reason
- **other**: different than the ones previously specified

For any subject discontinuing the study, the investigator will:

- ask the subject to undergo, as far as possible, a final medical visit (ETV) to examine the subject's health conditions and perform the required blood sampling for the laboratory assays. This examination will verify that all values tested at screening have remained within a clinically acceptable range (i.e. not clinically significant changes compared to screening)
- arrange for alternative medical care of the withdrawn subject, if necessary
- record the subject decision about the use of collected biological samples

- report in the CRF date and time of the last dose administration, and date and primary reason of study discontinuation
- record in the CRF any follow-up, if the subject is withdrawn for an AE

Discontinued subjects will not be replaced.

19.5 Study termination

The study will be considered terminated at the date of the last visit of the last subject or upon completion of any follow-up procedure described in protocol. The investigator and the sponsor have the right to discontinue the study at any time for reasonable medical and/or administrative reasons. As far as possible, this should occur after mutual consultation. Reasons for discontinuation have to be documented appropriately.

20 ADMINISTRATIVE PROCEDURES

20.1 Material supplied to the clinical centre

Beside IMP, the following study material will be supplied to the clinical centre:

- final version of the study protocol
- CRF for each subject plus some spare copies
- copy of the investigator's brochure (IB) relative to the IMP
- informed consent forms

Moreover, before the start of the study, the investigator will be provided with the following documents: CFDA GCP, ICH guidelines, confidentiality agreement (if applicable), protocol amendments (if any), declaration of Helsinki, insurance statement, SAE forms, financial agreement (if applicable), confidential subject identification code list form, drug accountability forms, investigator and study staff list form.

20.2 Protocol amendments

In order to obtain interpretable results, neither the investigator nor the sponsor will alter the study conditions agreed upon and set out in this protocol. Amendments should be made by mutual agreement between the investigator and the sponsor. Any amendment must be set out in writing, giving the reasons, and being signed by all concerned parties. The amendment becomes then part of the protocol.

20.3 Study documentation and record keeping

The investigator should ensure the accuracy, completeness, legibility, and timeliness of the data reported to the sponsor in the CRFs and in all required reports.

The investigator must keep source documents for each subject in the study. All information on the CRFs must be traceable to these source documents, which are generally stored in the subject's medical file. The source documents should contain all demographic and medical information, including laboratory data, ECGs, etc., and the original signed informed consent forms.

Data reported on the CRF that are derived from source documents should be consistent with the source documents or the discrepancies should be explained.

The investigator and the sponsor should maintain the study documents as specified in the "Essential Documents for the Conduct of a Clinical Trial" chapter 8 of ICH-GCP and as required by CFDA GCP and the applicable regulatory requirement(s).

These are documents which individually and collectively permit evaluation of a study and the quality of the data produced and include groups of documents, generated before the study commences, during the clinical study, and after termination of the study and include but are not limited to, study protocol, amendments, submission and approval of EC, raw data of subjects including lab tests and ECG tracing, insurance contracts, certificate of analysis of the

IMP(s), drug accountability records, signed informed consent forms, confidential subjects identification code, CRFs, curricula vitae of the investigator and other participants in the study, study staff lists and responsibilities, monitoring reports and final study report.

The investigator and the sponsor should take measures to prevent accidental or premature destruction of these documents.

Study documents must be retained by the investigator and the sponsor as long as needed to comply with ICH-GCP, CFDA GCP, national and international regulations. By signing the protocol, the investigator and the sponsor agree to adhere to these requirements.

20.4 Study subjects' recruitment

Study participants will be recruited from the volunteers' database. The study site may also use the site management organisation to recruit the study participants.

In addition, they may also use recruitment posters in other departments within the hospital, community outside or newspapers to raise awareness of the study that is going on.

Other departments within the hospital will be involved to recommend study participants as well.

The clinical site has detailed SOPs on the recruitment process.

20.5 Confidentiality and data protection

By signing this protocol, the investigator and the CRO agree to keep all the information provided by the sponsor in strict confidentiality and to request the same confidentiality from his/her staff. Study documents provided by the sponsor (protocols, IB, CRFs and other materials) will be stored appropriately to ensure confidentiality. The information provided by the sponsor to the investigator and to the CRO cannot be disclosed to others without direct written authorisation from the sponsor, except for the extent necessary to obtain the informed consent from the subjects wishing to participate in the study.

Data on subjects collected in the CRFs during the study will be documented in an anonymous way. If, as an exception, for safety or regulatory reasons identification of a subject becomes necessary, the monitor, the sponsor and the investigator will be bound to keep this information confidential.

20.6 Publication policy

The sponsor agrees that the study results (including negative and inconclusive as well as positive results) can be made publicly available by the investigator publishing in peer reviewed journals, presenting results at scientific congresses and posting information and results on internet-based public registers and databases.
Study results will be communicated in full to the competent Health Authorities by the submission of a complete clinical study report.

As the sponsor agrees that the study results can be published by the investigator(s), the investigator agrees to submit any manuscript (abstract, publication, paper, etc.) to the sponsor before any public disclosure.
This will be done in order to ensure that clinical study results are reported in an objective, accurate and balanced manner. The sponsor reviews the proposed manuscripts, before submission, within a reasonable period of time (30-90 days in relation with the complexity of the work).

The investigator will also be provided by the sponsor with the clinical study report and the results of any additional analysis, tables, figures, etc. undertaken for the purposes of the article, in order to take responsibility for the content of the publication(s).
On an exceptional basis, the sponsor may temporarily delay registration of certain data elements (e.g. compound, name, outcome, measures, etc.) to seek necessary intellectual property protection. This is because early disclosure of such data could, in some circumstances, prevent or negatively impact patentability.

21 STUDY RESPONSIBLE PERSONS

21.1 Sponsor

Zambon S.p.A., via Lillo del Duca 10, I-20091 Bresso, Italy
Phone: +39.02.66.52.41
Fax: +39.02.66.50.14.92

Protocol Review Committee Chairman

PPD

Sponsor's representatives

PPD

21.2 Drug assay

Eurofins Central Laboratory Shanghai, 395 Jiang Chang West Road, 7th Floor, Shanghai, 200436 China

21.3 Project management, data analysis & reporting, monitoring

PAREXEL International (IRL) Limited ("**PAREXEL**"), a corporation organized under the laws of Ireland with a registered address at 70 Sir John Rogerson's Quay, Dublin 2, Ireland.

22 REFERENCES

1. Ventresca GP, Cicchetti V, Ferrari V. Drugs in Bronchial Mucology. 1989. Cap. 4.1 Thiols 77-102. Edited by PC Braga and Allegra. Raven Press Ltd
2. Aruoma OI, Halliwell B, Hoey BM, Butler J. The antioxidant action of N-acetylcysteine: Its reaction with hydrogen peroxide, hydroxyl radical, superoxide and hypochlorous acid. Free Radical Biol Med 1988; 6:593-597
3. Ziment I. Acetylcysteine: a drug that is much more than a mucokinetic. Biomed & Pharmacother 1988; 42: 513-520
4. Borgstrom L, Kagedal B, Paulsen O. Pharmacokinetics of N-acetylcysteine in man. Eur J Clin Pharmacol 1986; 31: 217-222
5. Olsson B, Johansson M, Gabrielsson J, Bolme P. Pharmacokinetics and bioavailability of reduced and oxidized N-acetylcysteine. Eur J Clin Pharmacol 1988; 34: 77-82
6. Burgunder JM, Varriale A, Lauterburg BH. Effect of N-acetylcysteine on plasma cysteine and glutathione following paracetamol administration. Eur J Clin Pharmacol 1989; 36: 127-131
7. De Caro L, Ghizzi A, Costa R, Longo A, Ventresca GP, Lodola L. Pharmacokinetics and bioavailability of oral acetylcysteine in healthy volunteers. Arzneim-Forsh/Drug Res 1989; 39: 382-386
8. Borgstrom L, Kagedal B. Dose dependent pharmacokinetics of N-acetylcysteine after oral dosing to man. Biopharmaceutics & Drug Disposition 1990; 11: 131-136
9. Frascio M. Pharmacokinetic study after single dose (1, 2, and 4 g) of a new N-acetylcysteine (NAC) oral formulation. Zambon Group Internal Report, code ZPM/94/34
10. Crestani S. Open label, randomized, cross-over, pharmacokinetic study to assess the bioequivalence of two oral N-Acetylcysteine preparations: 600 mg film coated tablets versus 600 mg effervescent tablets. 2002 Report in Zambon Group's file
11. Rusca A. Study CRO-PK-14-286 – Sponsor code Z7169J02. Comparative bioavailability study of N-acetyl-cysteine (NAC) 600 mg uncoated tablets vs. NAC 600 mg film-coated tablets in healthy male and female volunteers. CROSS Research S.A., Switzerland 2014
12. Rusca A. Study CRO-PK-07-189 – Sponsor code 7169J01. Comparative bioavailability study of a new oral N-acetyl-cysteine (NAC) formulation vs. Fluimucil®, Zambon in healthy volunteers. CROSS Research S.A., Switzerland, 2008
13. Rusca A. Study CRO-PK-09-225 – Sponsor code 7203J01. Pilot bioavailability study of a new 1200 mg N-acetyl-cysteine (NAC) effervescent tablet formulation vs. Fluimucil® 600 mg effervescent tablet (Zambon S.p.A., Italy) in healthy male and female volunteers. CROSS Research S.A., Switzerland, 2010
14. Brown M, Bjorksten A, Medved I and M McKenna. Pharmacokinetics of intravenous N-acetylcysteine in men at rest and during exercise. Eur J Clin Pharmacol 2004;60:717-723
15. Jones AL, Jarvier D. Simpson D. Pharmacokinetics of N-acetylcysteine are altered in patients with chronic liver diseases. Aliment Pharmacol Ther. 1997; 787-791
16. Prescott LF, Donovan JW, Jarvie DR, Proudfoot AT. The disposition and kinetics of intravenous N-acetylcysteine in patients with paracetamol overdosage. Eur J Clin Pharmacol 1989; 37: 501-506
17. Pendyala L, Creaven PJ. Pharmacokinetic and pharmacodynamic studies of N-acetylcysteine, a potential chemopreventing agent during a phase I trial. Cancer Epidemiology, Biomarkers and Prevention 1995; 4: 245-251
18. Rodenstein D, De Coster A, Gazzaniga A. Pharmacokinetics of oral acetylcysteine: absorption, binding and metabolism in patients with respiratory disorders. Clinical Pharmacokinetics 1978; 3: 247-254
19. Pharmacokinetics of N-Acetylcysteine following repeated i.v. infusions to hemodialysed chronic renal failure patients. Internal Report 2002. Zambon Group's file

20. Sjodin K, Nilsson E, Hallemberg A, Tunek A. Metabolism of N-acetyl-l-cysteine Biochem. Pharmacol 38 1989; 22: 3981-3985
21. Locatelli C. Fluimucil 20% injectable (N-Acetylcysteine). Expert Report on the clinical documentation 1996. Zambon Group's file
22. N-acetyl-L-cysteine (NAC) intravenous Investigator's Brochure, edition number 1, 20 April 2018
23. Guidelines on GMP, published by the Commission in The rules governing medicinal products in the European Community, Volume 4
24. Guidance on the investigation of bioequivalence. CPMP/EWP/QWP/1401/98 Rev. 1/Corr **, 20 January 2010
25. U.S. Department of Health and Human Services and U.S. Department of Agriculture, Nutrition and your health: 2015-2020 Dietary Guidelines, Appendix 9

CLINICAL STUDY PROTOCOL

Sponsor code Z7244J01

Phase I study to evaluate pharmacokinetics, safety and tolerability of single and multiple i.v. doses of N-acetylcysteine (NAC) in Chinese healthy volunteers

Single and multiple dose, single centre, open-label, one-way, pharmacokinetics, safety and tolerability clinical trial

Test formulation:	NAC 300 mg/ 3 mL solution for injection, Zambon S.p.A., Italy.
Sponsor:	Zambon S.p.A., via Lillo del Duca 10, I-20091 Bresso, Italy Phone: +39.02.66.52.41 Fax: +39.02.66.50.14.92
Investigator:	PPD - Principal investigator Ruijin Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, Phase I Unit, No.197, Rui Jin Er Road, Shanghai China Email PPD
Development phase:	I
Version and date:	Final version 2.0, 19 Dec 2018

This study will be conducted in accordance with the current version of Good Clinical Practice (GCP), ICH topic E6 (R2 and CFDA GCP)

1 PROTOCOL APPROVAL

1. SPONSOR

Zambon S.p.A., Italy

Sponsor's representative

PPD

PPD

Date

PPD

Signature

2. INVESTIGATOR

Principal investigator

I have read this protocol and agree to conduct this study in accordance with all the stipulations of the protocol and in accordance with the Declaration of Helsinki, the current revision of Good Clinical Practice (GCP), ICH topic E6 (R2), and the applicable local law requirements.

PPD

PPD

Date

PPD

Signature

2 STUDY SYNOPSIS

Title: Phase I study to evaluate pharmacokinetics, safety and tolerability of single and multiple i.v. doses of N-acetylcysteine (NAC) in Chinese healthy volunteers

Protocol number: Z7244J01

Clinical phase: Phase I

Study design: Single and multiple dose, single centre, open-label, one-way, pharmacokinetics, safety and tolerability clinical trial

Planned nr. of centres / countries: 1/China

Investigator and centre: : Principal investigator: PPD Ruijin Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, Phase I Unit, No.197,Rui Jin Er Road, Shanghai, China

Investigational medicinal product (IMP):
Test product: NAC, 300 mg/ 3 mL solution for injection, Zambon S.p.A., Italy

Dose regimen:
Two ampoules (300 + 300 mg) corresponding to a total dose of 600 mg of NAC diluted in 10 mL of NaCl 0.9% saline solution, will be administered by a 5-minute intravenous (i.v.) infusion.
Single dose:
One (1) dose of the investigational product will be administered under fasting conditions on day 1 at 08:00 ±1 h
Multiple dose regime:
Five (5) doses of the investigational product will be administered twice a day (b.i.d.) on days 4 and 5 at 08:00 ±1 h and 20:00 ±1 h and one dose will be administered on day 6 at 08:00 ±1.

Objective: To evaluate the NAC pharmacokinetics, safety and tolerability after single and multiple dose i.v. administration.

End-points:
Primary end-point:
- To evaluate pharmacokinetic parameters of NAC in plasma after single and multiple dose administration of the investigational product.

Secondary end-points:
- To collect safety and tolerability data after single and multiple dose administration of the investigational product.

Study variables:
Primary variables:
After single dose:
- C_{max}: maximum NAC plasma concentration
- t_{max}: time to achieve C_{max}
- k_{el}: terminal elimination rate constant, calculated, if feasible, by log-linear regression using at least 3 points
- $t_{1/2}$: NAC half-life, calculated, if feasible, as $\ln 2/k_{el}$
- $AUC_{(0-t)}$: area under the concentration-time curve from single dose to the last observed concentration time t, calculated with the linear up/log down trapezoidal method
- $AUC_{(0-\infty)}$: area under the concentration-time curve extrapolated to infinity, calculated, if feasible, as $AUC_{(0-t)} + C_t/k_{el}$, where C_t is the last measurable drug concentration
- $AUC_{(0-12)}$: area under the concentration-time curve at steady-state in the tau interval (from single dose to 12 h post-dose), calculated with the linear up/log down trapezoidal method
- V_d: volume of distribution associated with the terminal slope, calculated, if feasible, as $Dose/(AUC_{(0-\infty)}*k_{el})$
- CL_t: total body clearance, calculated, if feasible, as $Dose/ AUC_{(0-\infty)}$
- $Ae_{(0-t)}$: total amount of NAC excreted in urine from single dose up to 32 h
- $Fe_{(0-t)}$: total fraction of NAC dose excreted in urine from single dose up to 32 h
- CL_r: renal clearance, calculated, if feasible, as $Ae_{(0-t)}/ AUC_{(0-\infty)}$

STUDY SYNOPSIS (cont.)

Study variables (continued):
After multiple dose:

- C_{ss_max}: maximum NAC plasma concentration at steady-state
- t_{ss_max}: time to achieve C_{ss_max}
- C_{ss_min}: trough NAC plasma concentration at steady-state, measured as concentration at t=12 h
- $AUCss_{(0-t)}$: area under the concentration-time curve at steady-state from the last multiple dose to the last observed concentration time t, calculated with the linear up/log down trapezoidal method
- $AUCss_{(0-12)}$: area under the concentration-time curve at steady-state in the tau interval (from the last multiple dose to 12 h post-dose), calculated with the linear up/log down trapezoidal method
- C_{ss_av}: average NAC plasma concentration at steady-state, calculated as $AUCss_{(0-12)}$ /tau
- R: accumulation ratio, calculated as $AUCss_{(0-12)}$/ $AUC_{(0-12)}$
- DF%: degree of fluctuation over one dosing interval at steady-state, calculated as $(C_{ss\_max} - C_{ss\_min})/ C_{ss\_av}*100$
- $Aess_{(0-t)}$: total amount of NAC excreted in urine from the last multiple dose to 32 h at steady-state

Secondary variables:

- Treatment emergent adverse events (TEAEs), vital signs (blood pressure, heart rate), body weight, physical examinations, clinical laboratory parameters.

Analytics: total NAC will be determined in plasma and urine at a certified bioanalytical laboratory to be designated, using a LC-MS/MS validated method with a Lower Quantification Limit (LQL) of 10 ng/mL. Analytical facilities and procedures are in compliance with the general principles of GLP regulations

Sample size: Twenty-four (24) healthy male and female Chinese volunteers will be included in the study. Drop-out subjects will not be replaced.

Main selection criteria:
Inclusion criteria:

1. *Informed consent*: signed written informed consent before inclusion in the study
2. *Ethnicity, Sex and Age*: Chinese males and females, 18-45 year old inclusive
3. *Weight:* body weight ≥50 kg;
4. *Body Mass Index*: 19-26 kg/m^2 inclusive
5. *Vital signs*: systolic blood pressure 100-139 mmHg, diastolic blood pressure 50-89 mmHg, heart rate 50-90 bpm, measured after 5 min at rest in the sitting/supine position (to be chosen according to the usual procedure at the clinical site)
6. *Full comprehension*: ability to comprehend the full nature and purpose of the study, including possible risks and side effects; ability to co-operate with the investigator and to comply with the requirements of the entire study
7. *Nicotine addiction (smoker subjects only)*: ability to abstain from smoking for the duration of the clinical study
8. *Contraception and fertility (women only)*: women of child-bearing potential must be using at least one of the following reliable methods of contraception:
   a. Hormonal oral, implantable, transdermal, or injectable contraceptives for at least 60 calendar days before the screening visit
   b. A non-hormonal intrauterine device or female condom with spermicide or contraceptive sponge with spermicide or diaphragm with spermicide or cervical cap with spermicide for at least 60 calendar days before the screening visit
   c. A male sexual partner who agrees to use a male condom with spermicide
   d. A sterile sexual partner

   Women of non-child-bearing potential or in post-menopausal status for at least 1 year will be admitted.
   Women of childbearing potential should be willing to adopt abstinence or contraception measures during the study and two weeks post-dose.
   For all women, pregnancy test result must be negative at screening and day -1.

STUDY SYNOPSIS (cont.)

Exclusion criteria:

1. *Electrocardiogram (12-lead ECG in supine position)*: clinically significant abnormalities
2. *Physical findings*: clinically significant abnormal physical findings which could interfere with the objectives of the study
3. *Laboratory analyses*: clinically significant abnormal laboratory values indicative of physical illness, in particular significant laboratory abnormality indicative of hepatic condition (more than 3 times the upper limit)
4. *Allergy*: ascertained or presumptive hypersensitivity to the active principle and/or formulations' ingredients; history of anaphylaxis to drugs or allergic reactions in general, which the investigator considers may affect the outcome of the study
5. *Diseases*: significant history of renal, hepatic, gastrointestinal, cardiovascular, respiratory, skin, haematological, endocrine, urologic, metabolic, neurological or psychiatric diseases, as determined by the investigator, that may interfere with the aim of the study; history of carcinoma *in situ* and malignant disease; active bacterial or viral infection and fever >38° C within 48 h prior to study treatment administration
6. *Virology*: positive result of HIV, hepatitis B (HBV), hepatitis C (HCV) or *Treponema pallidum* (TP) assays
7. *Surgery*: any surgery within 2 months of screening (excluding diagnostic surgery)
8. *Medications*: medications, including over the counter (OTC) medications, herbal remedies and traditional Chinese remedies for 2 weeks before the start of the study. Hormonal contraceptives for women will be allowed
9. *Investigative drug studies*: participation in the evaluation of any investigational product for 30 calendar days before this study
10. *Blood donation*: blood donations for 90 calendar days before this study
11. *Drug, alcohol, caffeine, tobacco*: history of drug, alcohol [>1 drink/day for females and >2 drinks/day for males, defined according to the USDA Dietary Guidelines 2015-2020] caffeine (>5 cups coffee/tea/day) or tobacco abuse (≥10 cigarettes/day)
12. *Abuse drug test*: positive urine abuse drug test at screening or day -1
13. *Alcohol test*: positive alcohol breath test at day -1
14. *Diet*: abnormal diets (<1600 or >3500 kcal/day) or substantial changes in eating habits in the 4 weeks before this study; vegetarians; consumption of alcohol, grapefruit, products containing grapefruit, or beverages containing xanthines (coffee, tea, soda, coffee with milk, energy drinks) within 48 hours prior to the enrolment
15. Pregnancy (females only): positive or missing pregnancy test at screening or day -1, pregnant or lactating women
16. Vaccination within 4 weeks of study treatment
17. Other unspecified reasons that, in the opinion of the investigator, make the subject unsuitable for enrolment.

Schedule:

	Day	Procedures/Assessments	Notes
Screening – Visit 1	*From day -14 to day -2*	➢ Explanation to the subject of study aims, procedures and possible risks ➢ Informed consent signature ➢ Screening number (e.g. [redacted], etc.) ➢ Demographic data and life style recording ➢ Medical/surgical history ➢ Previous/concomitant medications ➢ Full physical examination (body weight, height, vital signs, physical abnormalities) ➢ ECG recording ➢ Clinical laboratory analyses: haematology, blood chemistry, urinalysis, serum virology, bacteriology and pregnancy test (women) ➢ Urine multi-drug kit test ➢ Adverse event (AE) monitoring ➢ Inclusion/exclusion criteria evaluation ➢ Eligibility evaluation	The subject screening numbers will be in running order (e.g. [redacted], etc.).

STUDY SYNOPSIS (cont.)

Schedule (continued):

	Day	Procedures/Assessments	Notes
Visit 2	*Day -1*	➢ Alcohol breath test ➢ Urine multi-drug kit test ➢ Urine pregnancy test (women) ➢ Inclusion/exclusion criteria evaluation ➢ Eligibility evaluation ➢ Enrolment ➢ Subject study number will be the screening number if successfully enrolled (e.g. [redacted], etc.) ➢ Check of AEs and concomitant medications	Arrival at the clinic before the evening (before 18:30) Confinement until the morning of day 8 Standardised dinner Fasting for at least 10 h (overnight) before first dosing
Visit 3	*Day 1*	➢ Vital sign measurement at pre-dose ➢ IMP administration at 08:00 ± 1 h; intravenous infusion for 5 min ➢ Vital signs measurement at 1 h post-dose ➢ Blood sample collection for pharmacokinetic analysis at: pre-dose (0) and 5 (at the end of the infusion), 8, 12, 15, 20, 25, 30, 60 min, 2, 4, 6, 8, 10 and 12 h post-dose ➢ Urine sample collection for pharmacokinetic analysis at: 0-4; 4-8; 8-12 and 12-24 h post-dose ➢ Check of AEs and concomitant medications	Standardized lunch at 13:00 standardized dinner at 21:00
	Day 2	➢ Blood sample collection for pharmacokinetic analysis at 24 and 32 h post-dose ➢ Urine sample collection for pharmacokinetic analysis at 24-32 h post-dose ➢ Check of AEs and concomitant medications	Standardized breakfast around 9:00 Standardized lunch around 13:00 Standardized dinner around 21:00
	Day 3	➢ Vital sign measurement at 48 h post-dose ➢ Physical examination (body weight, physical abnormalities) at 48 h post-dose ➢ Clinical laboratory analyses: haematology, blood chemistry, urinalysis at 48 h post-dose ➢ Check of AEs and concomitant medications	Standardized breakfast around 9:00 Standardized lunch around 13:00 Standardized dinner around 21:00
Visit 4	*From day 4 to day 5*	➢ IMP administration at 08:00 ± 1 h; infusion for 5 min ➢ IMP administration at 20:00 ± 1 h; infusion for 5 min ➢ Blood sample collection for pharmacokinetic analysis at: pre-dose (0) before the morning infusion ➢ Check of AEs and concomitant medications	Standardized breakfast around 9:00 Standardized lunch around 13:00 Standardized dinner around 21:00

STUDY SYNOPSIS (cont.)

Schedule (continued):

	Day	Procedures/Assessments	Notes
Visit 5	*Day 6*	➢ Vital sign measurement at pre-dose ➢ IMP administration at 08:00 ± 1 h; infusion for 5 min ➢ Vital signs measurement at 1 h post-dose ➢ Blood sample collection for pharmacokinetic analysis at: pre-dose (0) and 5 (at the end of the infusion), 8, 12, 15, 20, 25, 30, 60 min, 2, 4, 6, 8, 10 and 12 h post-dose ➢ Urine sample collection for pharmacokinetic analysis at: 0-4; 4-8; 8-12 and 12-24 h post-dose ➢ Check of AEs and concomitant medications	Standardized lunch at 13:00 (about 5 h post-dose) Standardized dinner at 21:00 (about 13 h post-dose)
	Day 7	➢ Blood sample collection for pharmacokinetic analysis at 24 and 32 h post-dose ➢ Urine sample collection for pharmacokinetic analysis at 24-32 h post-dose ➢ Check of AEs and concomitant medications	Standardized breakfast around 9:00 Standardized lunch around 13:00 Standardized dinner around 21:00
Final Visit/ETV	*Day 8 or early termination visit (ETV) in case of discontinuation*	➢ Vital sign measurement at 48 h post-dose (or at ETV) ➢ Physical examination (body weight, physical abnormalities) ➢ Clinical laboratory analyses: haematology, blood chemistry, urinalysis ➢ Check of AEs and concomitant medications In case of clinically significant results at the final visit, the subjects will be followed-up by the investigator until the normalisation of the concerned clinical parameter(s)	Discharge from the clinical centre in the morning of Day 8 after vital sign measurement, blood sampling for clinical laboratory assays and physical examination or in case of ETV. Upon leaving, the subjects will be instructed to contact immediately the investigator in case of occurrence of any untoward medical occurrence

Life style and constraints:
During the study, the subjects will be confined from the evening preceding the first drug administration (study day -1) until the morning of day 8 (after the final visit).
The subjects will not take any food or drinks (except water) for at least 10 h (i.e. overnight) before the first drug administration (Day 1) and will remain fasted up to 5 h post-dose.
During the study, water will be allowed as desired. Coffee, tea or food containing xanthines (i.e. coffee, tea, soda, coffee with milk, energy drinks coke, chocolate, etc.), alcohol and grapefruit will be forbidden starting 48 h prior to the enrolment until the end of the study. Smoking is not allowed for the whole study duration.

STUDY SYNOPSIS (cont.)

Withdrawal of subjects:
It will be documented whether or not each subject completes the clinical study. In case of premature discontinuation of any subject, the primary reason for discontinuation will be recorded.

- Adverse event: Any (significant) adverse event that in the opinion of the investigator or concerned subject is not compatible with study continuation
- death: the absence of life or state of being dead
- lost to follow-up: the loss or lack of continuation of a subject up to follow-up
- non-compliance with study drug: an indication that a subject has not agreed with or followed the instructions related to the study medication
- physician decision: a position, opinion or judgment reached after consideration by a physician with reference to the subject
- pregnancy: pregnancy is the state or condition of having a developing embryo or fetus in the body (uterus), after union of an ovum and spermatozoon, during the period from conception to birth
- protocol deviation: an event or decision that stands in contrast to the guidelines set out by the protocol
- study terminated by sponsor: an indication that a clinical study was stopped by its sponsor
- technical problems: a problem with some technical aspect of a clinical study, usually related to an instrument
- withdrawal by subject: study discontinuation requested by a subject for whatever reason

For any subject discontinuing the study, the investigator will ask the subject to undergo, as far as possible, a final medical visit (ETV) to examine the subject's health conditions and perform the required blood sampling for the laboratory assays. This examination will verify that all values tested at screening have remained within a clinically acceptable range (i.e. not clinically significant changes compared to screening) and report in the case report form (CRF) date and time of the last dose administration, and date and primary reason of study discontinuation.

Data analysis:
The data documented in this trial and the measured clinical parameters will be presented using classic descriptive statistics (i.e. total number of subjects treated [N], number of observations [n], mean standard deviation [SD], minimum [Min], median, maximum [Max]) for quantitative variables and frequencies (i.e. count and percentages) for qualitative variables if not stated otherwise.

Analysis set:
Enrolled set: all enrolled subjects. This analysis set will be used for demographic, baseline and background characteristics
Safety set: all subjects who receive at least one dose of IMP. This analysis set will be used for the safety analyses
PK set: all enrolled subjects who fulfil the study protocol requirements in terms of IMP administration and have evaluable pharmacokinetic data readouts for the planned analysis, with no major deviations that may affect the pharmacokinetic results. This analysis set will be used for the statistical analysis of the pharmacokinetic results

Safety Assessments:
The statistical analysis of demographic and safety data will be performed using the analysis software SAS® .
The toxicity grading of laboratory tests is determined based on the NCI CTCAE V4.03. All AEs, adverse drug reactions and serious adverse events will be summarised by system organ class, severity and relationship to study drug.

Pharmacokinetics:
The statistical analysis of PK parameters will be performed using the validated software Phoenix® WinNonlin® version 6.3 or higher (Certara USA, Inc., Princeton, NJ) (actual version will be stated in the final report).
The individual plasma/urine concentration and pharmacokinetic parameters will be presented in listings and their descriptive statistics summarised in tables.

STUDY SYNOPSIS (cont.)

Background:

N-acetyl-L-cysteine (NAC) is the N-acetyl derivative of the naturally occurring amino acid L-cysteine.

NAC was introduced into the Italian pharmaceutical market in 1965 and it is now manufactured and marketed by Zambon S.p.A. in Europe, Asia, South America, Central America, and the Middle East, under various trade names (Ventresca 1989).

NAC is widely known as a mucolytic agent, with a well-established use in the treatment of respiratory tract disorders. However, after more than 35 years from its introduction in the clinical practice, this molecule continues to be the object of scientific interest due to its potential in the treatment of several conditions other than those related to the respiratory system. NAC can also act as a direct antioxidant agent due to the -SH group (Auroma 1988) and can easily penetrate into the cells where it is deacetylated to L-cysteine, thus supporting the biosynthesis of glutathione (Ziment 1988).

Clinical pharmacology and pharmacokinetics

Several pharmacokinetic (PK) studies were carried out in healthy volunteers (Borgstrom 1986, Olsson 1988, Burgunder 1989, De Caro 1989, Borgstrom 1990, Frascio, Crestani 2002, Rusca 2014, Rusca 2010, Rusca 2002). Further PK studies were carried out in patients with chronic liver damage (Jones 1997), with liver damage caused by paracetamol overdose (Prescott 1989), in subjects under long-term treatment for chemoprevention (Pendyala 1995), in patients with respiratory disorders (Rodenstein 1978) and in patients with End Stage Renal Diseases (Internal report 2002).

In the clinical studies, NAC was administered orally and intravenously both as single and repeated dose. Oral NAC was administered in different dosage forms, such as capsules, granulate, effervescent tablets, fast dissolving tablets, slow-release tablets.

Clinical trials and safety concerns

NAC has been available to patients as a mucolytic agent for many years and has a well-established and satisfactory safety and tolerability profile (see IB).

Independently of route of administration (inhalation, oral, intravenous) adverse events are reported rarely following NAC administration and are usually mild and transient.

The most frequent adverse events associated with NAC oral administration are gastrointestinal (e.g., vomiting, diarrhoea, stomatitis, abdominal pain and nausea). Hypersensitivity reactions (e.g. anaphylactoid reactions, urticaria, rash, pruritus, and bronchospasm) have been reported less frequently after oral administration, while with the IV administration they were reported more frequently compared to gastrointestinal symptoms. Some of the hypersensitivity reactions were associated to higher infusion rate. Most anaphylactoid reactions can be managed by temporarily suspending the acetylcysteine infusion, administering appropriate supportive care. A cumulative analysis of all safety reports received by the Sponsor up to September 2017 for NAC IV, confirmed the nature and severity of the events were in line with the above profile of the product.

Intravenous NAC has been studied in clinical trials and published case series both as mucolytic agent and as an antidote against intoxication by paracetamol and other toxic agents including *Amanita phalloides* and chlorinated hydrocarbons. Overall, in published studies in humans, a total of 745 patients were exposed to i.v. NAC at doses ranging from 500 mg/day to 980mg/kg/day for a duration of 1 to 18 days. Details of safety results are included in the IB.

In published studies, whereas i.v. NAC as mucolytic agent typically was given at doses up to 1000 mg/day, much higher i.v. doses of NAC as antidote were given over multiple days, maintaining a satisfactory safety profile.

Locatelli and colleagues published two case series, one of 86 patients with acute intoxication from *Amanita phalloides* and the other of 35 patients with acute intoxication from chlorinated hydrocarbons. Patients received i.v. NAC 300 mg/kg/day for 3 to 14 days and 3 to 18 days respectively. The safety and tolerability profile were overall benign, with 4 out of 121 exposed patients suffering from anaphylactoid reaction or rash (due to the rapid administration of the bolus according to the authors), none of which caused discontinuation of treatment. No other AEs were reported. (Locatelli 1996).

Rationale

NAC is widely known as a mucolytic agent, with a well-established use in the treatment of respiratory tract disorders. In the present study, the kinetic profile and the bioavailability of NAC will be investigated in Chinese healthy male and female volunteers all receiving the same single i.v. dose, i.e. a total dose of 600 mg of NAC, and the same multiple dose administrations, i.e. five i.v. doses of 600 mg of NAC administered b.i.d. for 2 days and once on the last day. The safety and tolerability will be monitored as well.

The present study will be part of the clinical development plan of NAC i.v. for registration in China of this formulation.

3 STUDY SCHEDULE

ACTIVITIES	Screening	Single Dose				Multiple Dose				Final visit/ETV[1]
Visit	V1	V2	V3			V4		V5		
	Day -14/-2	Day -1	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8[2]
Informed consent	x									
Demography and lifestyle	x									
Medical and surgical history	x									
Physical examination	x				x					x
Prior and concomitant medications	x	x	x	x	x	x	x	x	x	x
Height	x									
Body Weight	x				x					x
Clinical laboratory analysis (haematology, blood chemistry, urinalysis)	x				x					x
Virology and bacteriology	x									
Serum pregnancy test (women)	x									
Urine multi-drug kit test	x	x								
Blood pressure and heart rate	x		x[3]		x			x[3]		x[4]
Alcohol breath test		x								
Urine pregnancy test (women)		x								
ECG	X									
Inclusion/exclusion criteria	X	x								
Subject eligibility	X	x								
Enrolment		x								
Confinement		x	x	x	x	x	x	x	x	
Discharge										x
Investigational product administration			x[5]			x[6]	x[6]	x[5]		
Blood sampling			x[7]	x[7]		x[8]	x[8]	x[7]	x[7]	
Urine sampling			x[9]	x[9]				x[9]	x[9]	
Standardised meals		x[10]	x[11]	x[12]	x[12]	x[12]	x[12]	x[11]	x[12]	
Adverse event monitoring[13]	x	x	x	x	x	x	x	x	x	x

1. *Early termination visit (ETV)*
2. *Final visit on day 8 or in case of ETV*
3. *At pre-dose and 1 h post-dose*
4. *The vital signs check at 48 h post-dose (day 8), will correspond to the final measurement*
5. *At 8:00 ± 1 h: 5-min infusion*
6. *At 8:00 ± 1 h and at 20:00 ± 1 h; 5-min infusion*
7. *At pre-dose (0) and 5 (at the end of the infusion), 8, 12, 15, 20, 25, 30, 60 min and 2, 4, 6, 8, 10, 12, 24 and 32 h post-dose*
8. *At pre-dose (0)*
9. *0-4; 4-8; 8-12, 12-24 and 24-32 h post-dose*
10. *Standardised dinner*
11. *Standardised lunch and dinner*
12. *Standardised breakfast, lunch and dinner*
13. *Adverse events monitored starting at the screening visit, immediately after informed consent, up to the final visit/ETV*

4 TABLE OF CONTENTS

4.1 TABLES

Page

5 LIST OF ABBREVIATIONS

β-HCG	human chorionic gonadotropin β
γ-GT	γ-Glutamyl transpeptidase
ADR	Adverse Drug Reaction
AE	Adverse Event
ALT	Alanine aminotransferase
Ae_{0-t}	Total amount of NAC excreted in urine
$Aess_{(0-t)}$	Total amount of NAC excreted in urine at steady-state
ANOVA	Analysis of Variance
AST	Aspartate aminotransferase
BB	Blue book
BLQL	Below Lower Quantification Limit
BUN	Blood Urea Nitrogen
$AUC_{(0-t)}$	Area under the concentration-time curve from single dose to the last observed concentration time t
$AUC_{(0-\infty)}$	Area under the concentration-time curve extrapolated to infinity
$AUC_{(0-12)}$	Area under the concentration-time curve at steady-state in the tau interval
$AUCss_{(0-t)}$	Area under the concentration-time curve at steady-state from the last multiple dose to the last observed concentration time t
$AUCss_{(0-12)}$	Area under the concentration-time curve at steady-state in the tau interval
BMI	Body Mass Index
BP	Blood Pressure
CA	Competent Authority
CDISC	Clinical Data Interchange Standards Consortium
CFDA	China Food And Drug Administration
CI	Confidence Interval
C_{max}	Peak drug concentration
CLt	Total body clearance
CLr	Renal clearance
CPL	Clinical Project Leader
CRA	Clinical Research Associate
CRF	Case Report Form
CRO	Contract Research Organisation
CS	Clinically Significant
CSP	Clinical Study Protocol
CSR	Clinical Study Report
C_{ss_av}	Average NAC plasma concentration at steady-state
C_{ss_max}	Maximum plasma concentration at steady-state
C_{ss_min}	Trough plasma concentration at steady-state
CV	Coefficient of Variation
DBP	Diastolic Blood Pressure
DF	Degree of fluctuation
DSU	Drug safety unit
DTT	Dithiothreitol
EC	Ethics Committee
ECG	Electrocardiogram
ETV	Early Termination Visit
FDA	Food and Drug Administration
Fe_{0-t}	Total fraction of NAC dose excreted in urine
F_{rel}	Relative Bioavailability
FSFV	First Subject First Visit
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
HBs Ag	Hepatitis B virus surface antigen
HCV Ab	Hepatitis C virus antibodies
HIV	Human Immunodeficiency Virus

HR	Heart Rate
IB	Investigator's Brochure
ICH	International Conference on Harmonisation
IRB/IEC	Institutional Review Board/Independent Ethics Committee
i.m.	Intramuscular
IMP	Investigational Medicinal Product
IUD	Intra-Uterine Device
i.v.	Intravenous
k_{el}	Terminal elimination rate constant
LQL	Lower Quantification Limit
LSLV	Last Subject Last Visit
MCH	Mean Cell Haemoglobin
MCHC	Mean Cell Haemoglobin Concentration
MCV	Mean Cell Volume
MedDRA	Medical Dictionary for Regulatory Activities
MRT	Mean Residence Time
MW	Molecular Weight
N	Normal
NA	Not Applicable
NAC	N-acetyl-L-cysteine
NC	Not calculated
NCS	Not clinically significant
NSAIDs	Non-Steroidal Anti-Inflammatory Drugs
OTC	Over The Counter
PE	Point Estimate
PK	Pharmacokinetics
PT	Preferred Term
PTAE	Pre-Treatment Adverse Event
R	Accumulation ratio
RBC	Red Blood Cells
RSI	Reference safety information
SAE	Serious Adverse Event
SBP	Systolic Blood Pressure
SD	Standard Deviation
SOC	System Organ Class
SOP	Standard Operating Procedure
SDTM	Study Data Tabulation Model
SUSAR	Suspected Unexpected Serious Adverse Reaction
T	Test
TEAE	Treatment-Emergent Adverse Event
THC	delta-9-tetrahydrocannabinol
$t_{1/2}$	Half-life
t_{max}	Time to achieve C_{max}
t_{ss_max}	Time to achieve C_{ss_max}
USDA	United States Department of Agriculture
Vd	Volume of distribution
WBC	White Blood Cells
WHODDE	World Health Organisation Drug Dictionary Enhanced

6 INTRODUCTION

6.1 Background

N-acetyl-L-cysteine (NAC) is the N-acetyl derivative of the naturally occurring amino acid L-cysteine.

NAC was introduced into the Italian pharmaceutical market in 1965 and it is now manufactured and marketed by Zambon S.p.A. in Europe, Asia, South America, Central America, and the Middle East, under various trade names (1).

NAC is widely known as a mucolytic agent, with a well-established use in the treatment of respiratory tract disorders. However, after more than 35 years from its introduction in the clinical practice, this molecule continues to be the object of scientific interest due to its potential in the treatment of several conditions other than those related to the respiratory system. NAC can also act as a direct antioxidant agent due to the -SH group (2) and can easily penetrate into the cells where it is deacetylated to L-cysteine, thus supporting the biosynthesis of glutathione (3).

6.2 Clinical pharmacology and pharmacokinetics

Several pharmacokinetic (PK) studies were carried out in healthy volunteers (4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14). Further PK studies were carried out in patients with chronic liver damage (15), with liver damage caused by paracetamol overdosage (16), in subjects under long-term treatment for chemoprevention (17), in patients with respiratory disorders (18) and in patients with End Stage Renal Diseases (19).
In the clinical studies, NAC was administered orally and intravenously both as single and repeated doses. Oral NAC was administered in different dosage forms, such as capsules, granulate, effervescent tablets, fast dissolving tablets, slow-release tablets.

In particular, Borgström *et al*. (4) studied for the first time the PK profile of NAC after i.v. dose of 600 mg of NAC infused over 5 min in 10 healthy male and female volunteers in comparison with 3 other oral dosage forms. The PK parameters obtained by the authors after i.v. infusion are summarised in the following table.

Table 6.2.1 PK parameters of plasma free NAC (N=10)

C_{max} (µmol/L)	t_{max} (h)	CL (L/kgxh)	CL_r (L/kgxh)	$t_{½}$ (h)	Ae_{0-12} (% of dose)
16.0±7.9	*0.65±0.33*	*0.207±0.017*	*0.058±0.011*	*2.27±0.32*	*29.0±3.2**

*mean±SD is reported; *: N=9; Source: 4*

However, the authors analysed NAC in deproteinised plasma thus missing the protein-bound NAC in their measurements.

Olsson *et al*. (5) improved the bioanalytical method and were able to measure the total NAC by reduction of the disulphide bonds in plasma before precipitating proteins. After single i.v. dose of 200 mg of NAC to 6 healthy volunteers, the authors found the following PK parameters:

Table 6.2.2 PK parameters of plasma total NAC (N=6)

	C_{max} (µM)	V_{ss} (L/kg)	CL (L/kgxh)
T	*121 (82.9 – 162)*	*0.47 (0.46 – 0.55)*	*0.11 (0.09 – 1.13)*

median (range) is reported; Source: 5

Jones *et al*. (15) studied the bioavailability of NAC after single i.v. dose of 600 mg of NAC infused over 3 min to patients with chronic liver disease, but also to 6 healthy male and female volunteers as control subjects. The authors found the following PK parameters for total NAC:

Table 6.2.3 PK parameters of plasma total NAC (N=6)

AUC (mg/Lxh)	CL_r (L/h)	$t_{½}$ (h)	Vd_{ss} (L)
93.9±9.6	*6.5±0.8*	*2.6±0.3*	*17.4±2.8*

mean±SD is reported; Source: 15

Brown *et al*. (14) investigated the PK of NAC infused i.v. to 24 healthy men at rest and during exercise. NAC was infused at the dose of 125 mg/kgxh for 15 min followed by 25 mg/kgxh for 35 min to healthy men at rest. Then, the infusion continued during exercise until fatigue. The authors found NAC peak concentrations in plasma at the end of the initial loading infusion. Mean peak concentration of total NAC was 205.1±68.3 mg/L, while mean CL was 0.164 L/kgxh.

It is worth noting that, while scientific literature shows that PK profiles of NAC after oral administration are very consistent (in terms of half-life, rate and extent of exposure, bioavailability, dose-linearity, etc.) among various publications, this is not the case for the data obtained after i.v. dosing. In fact, for this administration route, published studies differ in several key aspects, i.e., for example, type of administration, applied bioanalytical methods, drug doses, calculation methods for PK parameters. Furthermore, the number of subjects enrolled in each published study is very small. For these reasons, the comparability of the literature data is low among the published studies and potentially also to the expected results of the present study.

6.3 Clinical trials and safety concerns

NAC has been available to patients as a mucolytic agent for many years and has a well-established and satisfactory safety and tolerability profile (see IB).
Independently of route of administration (inhalation, oral, intravenous) adverse events are reported rarely following NAC administration and are usually mild and transient.
The most frequent adverse events associated with NAC oral administration are gastrointestinal (e.g., vomiting, diarrhoea, stomatitis, abdominal pain and nausea). Hypersensitivity reactions (e.g. anaphylactoid reactions, urticaria, rash, pruritus, and bronchospasm) have been reported less frequently after oral administration, while with the i.v. administration they were reported more frequently compared to gastrointestinal symptoms. Some of the hypersensitivity reactions were associated to higher infusion rate. Most anaphylactoid reactions can be managed by temporarily suspending the acetylcysteine infusion, administering appropriate supportive care.

A cumulative analysis of all safety reports received by the Sponsor up to September 2017 for NAC IV, confirmed the nature and severity of the events were in line with the above profile of the product.
Intravenous NAC has been studied in clinical trials and published case series both as mucolytic agent and as an antidote against intoxication by paracetamol and other toxic agents including *Amanita phalloides* and chlorinated hydrocarbons. Overall, in published studies in humans, a total of 745 patients were exposed to i.v. NAC at doses ranging from 500 mg/day to 980mg/kg/day for a duration of 1 to 18 days. Details of safety results are included in the IB.
In published studies, whereas i.v. NAC as mucolytic agent typically was given at doses up to 1000 mg/day, much higher i.v. doses of NAC as antidote were given over multiple days, maintaining a satisfactory safety profile.
Locatelli and colleagues published two case series, one of 86 patients with acute intoxication from *Amanita phalloides* and the other of 35 patients with acute intoxication from chlorinated hydrocarbons. Patients received i.v. NAC 300 mg/kg/day for 3 to 14 days and 3 to 18 days respectively. The safety and tolerability profile were overall benign, with 4 out of 121 exposed patients suffering from anaphylactoid reaction or rash (due to the rapid administration of the bolus according to the authors), none of which caused discontinuation of treatment. No other AEs were reported (21).

6.4 Rationale

NAC is widely known as a mucolytic agent, with a well-established use in the treatment of respiratory tract disorders.
In the present study, the kinetic profile and the bioavailability of NAC will be investigated in Chinese healthy male and female volunteers all receiving the same single i.v. dose, i.e. a total dose of 600 mg of NAC, and the same multiple dose administrations, i.e. five i.v. doses of 600 mg of NAC administered b.i.d. for 2 days and once on the last day. The safety and tolerability will be monitored as well.
The present study will be part of the clinical development plan of NAC i.v. for registration in China of this formulation.

6.5 Risk and benefits

NAC is a well-known drug which has been used for decades.

Undesired effects which may occur during treatment with NAC include: anaphylactic shock, anaphylactic reaction, anaphylactoid reaction, hypersensitivity, tachycardia, bronchospasm, dyspnoea, vomiting, nausea, angioedema, urticaria, flushing rash, pruritus, face oedema, blood pressure decreased, prothrombin time prolonged (for details refer to IB; 22).

Blood sampling with cannula insertion may cause minor discomfort. The risks associated with blood draws include pain, bleeding and bruising.

No specific benefits for the participants in the current study are foreseen. Their remuneration will be paid after study completion. The remuneration covers loss of time and any inconvenience caused by the participation in the study.

7 STUDY OBJECTIVES

The objective of the study is to evaluate the NAC pharmacokinetics, safety and tolerability after single and multiple dose i.v. administration.

7.1 Primary end-point

- To evaluate pharmacokinetic parameters of NAC in plasma after single and multiple dose administration of the investigational product.

7.2 Secondary end-point

- To collect safety and tolerability data after single and multiple dose administration of the investigational product.

8 CLINICAL SUPPLIES

8.1 Treatment

All the subjects enrolled in the study will receive the same treatment with the investigational medicinal product (IMP), i.e. NAC, 300 mg/ 3 mL solution for injection, as follows:

- on day 1 at 08:00 ±1 h, one dose of 600 mg of NAC (300 + 300 mg ampoule) will be administered under fasting conditions;

After a wash-out of 3 days:

- on days 4 and 5 at 08:00 ±1 h and 20:00 ±1 h and at 08:00 ±1 on day 6, 5 doses of 600 mg of NAC (300 + 300 mg ampoule) will be administered.

8.1.1 Description of products

8.1.1.1 Test product

IMP	NAC 300 mg/ 3 mL solution for injection
Active substance	N-acetyl-L-cysteine
Manufacturer (active substance)	F.I.S. Fabbrica Italiana Sintetici S.p.A., Via Dovaro, 36045 Lonigo (Vicenza), Italy (GMP compliant)
Manufacturer (finished product)	Zambon S.p.A., Via della Chimica 9, 36100 Vicenza, Italy (GMP compliant)
Pharmaceutical form	Solution for injection
Dose	300 mg/ 3 mL
Administration route	Parenteral

The analytical certificates will be supplied with the IMP. Quali-quantitative formulation is as follows:

Each 3 mL vial (10%) contains:
- N-acetyl-L-cysteine (NAC) 300 mg
- sodium hydroxide 74 mg
- disodium edetate 3 mg
- water for injections q.s. to 3 mL

8.1.2 Dose regimen

Two ampoules of IMP (300 + 300 mg) corresponding to a total dose of 600 mg of NAC diluted in 10 mL of NaCl 0.9% sterile saline solution, will be administered by a 5-minute i.v. infusion.

Single dose:

One (1) dose of IMP will be administered under fasting conditions on day 1 at 08:00 ±1 h.

Multiple dose regime:

Five (5) doses of IMP will be administered twice a day (b.i.d.) on days 4 and 5 at 08:00 ±1 h and 20:00 ±1 h and one dose will be administered on day 6 at 08:00 ±1.

8.1.3 Route and method of administration

Each dose of IMP will be prepared by diluting the content of 2 ampoules of IMP in 10 mL of saline, as described above (§ 8.1.2), and administered to each study subject at the clinical site only by the investigator or his/her deputy.

Each dose will be infused intravenously over 5 min.

The start of infusion (not the end) will be considered as time 0.

8.1.4 Investigational product distribution

The IMP will be exclusively used for the present clinical study and will only be administered to the subjects enrolled in the study.

8.2 Packaging and labelling

The IMP primary packaging will be glass vials.

The formulation labelling will report all the information requested according to the Annex 13 to the Good Manufacturing Practice (published by the Commission in The rules governing medicinal products in the European Community, Volume 4; 23) and in compliance with applicable laws and regulations as follows:

a. Name, address and telephone number of the sponsor, contract research organisation or investigator (the main contact for information on the product, clinical study and any emergency)

b. Pharmaceutical dosage form, route of administration, quantity of dosage units, and the name and strength

c. The batch and/or code number to identify the contents and packaging operation

d. A study reference code allowing identification of the study, site, investigator and sponsor if not given elsewhere

e. The study subject identification number/treatment number and where relevant, the visit number

f. The name of the investigator (if not included in (a) or (d))

g. Directions for use (reference may be made to a leaflet or other explanatory document intended for the study subject or person administering the product)

h. "For clinical study use only" or similar wording

i. The storage conditions

j. Period of use (use-by date, expiry date or re-test date as applicable), in month/year format and in a manner that avoids any ambiguity

k. "Keep out of reach of children"

Labels will be in local language.

8.3 Storage conditions

The IMP will be stored at ≤25° C in a dry locked place, sheltered from light.

8.4 Drug accountability

The IMP will be provided directly to the investigator by the Sponsor or designee, in excess of the amount necessary for the study (at least 25% excess).

After receipt of the IMP supply, the pharmacist will confirm in writing by signing and dating standard drug accountability forms.

At the end of the study, used, unused and partially used supplies of IMP provided by the Sponsor or designee will either be destroyed on site (upon written authorisation) or returned to the Sponsor or designee, after assessment of drug accountability.

9 INVESTIGATIONAL PLAN

9.1 Overall study design

This is a single and multiple dose, single centre, open-label, one-way, pharmacokinetics, safety and tolerability clinical trial of Phase I to be performed in Chinese healthy male and female volunteers.

9.2 Discussion of design

The study has been designed in agreement with the Chinese Technical Guideline on Clinical Pharmacokinetic Research of Chemical Drugs, 18 March 2005 and the European Guideline on the Investigation of Bioequivalence (CPMP/QWP/EWP/1401/98 Rev. 1, 20 January 2010) (24).

An open-label design is used since the primary end-point of the study is based on objective measurements of NAC in blood. The outcome variables are not influenced by the subjects or investigator being aware of the administered products.

Blood sampling time-points were selected on the basis of the known PK profile of NAC (4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14).

The dose of 600 mg has been selected since this is the efficacious, safe and well tolerated dose used in clinical practice (see also 22).

The bioanalysis will be performed in compliance with GCP and CFDA GCP regulations and in accordance with the applicable principles of GLP, as defined by OECD, in a GLP compliant facility. Moreover, sample analysis will be conducted in compliance with the Chinese Guidance on Bioanalysis: method validation and analysis of study samples (2015).

10 STUDY POPULATION

10.1 Target population

Chinese healthy male and female volunteers will be included in the study.

10.2 Inclusion criteria

To be enrolled in this study, subjects must fulfil all these criteria:

1. *Informed consent*: signed written informed consent before inclusion in the study
2. *Ethnicity, Sex and Age*: Chinese males and females, 18-45 year old inclusive
3. *Weight:* body weight ≥50 kg;
4. *Body Mass Index*: 19-26 kg/m^2 inclusive
5. *Vital signs*: systolic blood pressure 100-139 mmHg, diastolic blood pressure 50-89 mmHg, heart rate 50-90 bpm, measured after 5 min at rest in the sitting/supine position (to be chosen according to the usual procedure at the clinical site)
6. *Full comprehension*: ability to comprehend the full nature and purpose of the study, including possible risks and side effects; ability to co-operate with the investigator and to comply with the requirements of the entire study
7. *Nicotine addiction (smoker subjects only)*: ability to abstain from smoking for the duration of the clinical study
8. *Contraception and fertility (women only)*: women of child-bearing potential must be using at least one of the following reliable methods of contraception:
    a. Hormonal oral, implantable, transdermal, or injectable contraceptives for at least 60 calendar days before the screening visit
    b. A non-hormonal intrauterine device or female condom with spermicide or contraceptive sponge with spermicide or diaphragm with spermicide or cervical cap with spermicide for at least 60 calendar days before the screening visit
    c. A male sexual partner who agrees to use a male condom with spermicide
    d. A sterile sexual partner

    Women of non-child-bearing potential or in post-menopausal status for at least 1 year will be admitted.

    Women of childbearing potential should be willing to adopt abstinence or contraception measures during the study and two weeks post-dose.

    For all women, pregnancy test result must be negative at screening and day -1.

10.3 Exclusion criteria

Subjects meeting any of these criteria will not be enrolled in the study:

1. *Electrocardiogram (12-lead ECG in supine position)*: clinically significant abnormalities
2. *Physical findings*: clinically significant abnormal physical findings which could interfere with the objectives of the study
3. *Laboratory analyses*: clinically significant abnormal laboratory values indicative of physical illness, in particular significant laboratory abnormality indicative of hepatic condition (more than 3 times the upper limit)
4. *Allergy*: ascertained or presumptive hypersensitivity to the active principle and/or formulations' ingredients; history of anaphylaxis to drugs or allergic reactions in general, which the investigator considers may affect the outcome of the study
5. *Diseases*: significant history of renal, hepatic, gastrointestinal, cardiovascular, respiratory, skin, haematological, endocrine, urologic, metabolic, neurological or psychiatric diseases, as determined by the investigator, that may interfere with the aim of the study; history of carcinoma *in situ* and malignant disease; active bacterial or viral infection and fever >38° C within 48 h prior to study treatment administration
6. *Virology*: positive result of HIV, hepatitis B (HBV), hepatitis C (HCV) or *Treponema pallidum* (TP) assays
7. *Surgery*: any surgery within 60 calendar days of screening (excluding diagnostic surgery)
8. *Medications*: medications, including over the counter (OTC) medications, herbal remedies and traditional Chinese remedies for 2 weeks before the start of the study. Hormonal contraceptives for women will be allowed
9. *Investigative drug studies*: participation in the evaluation of any investigational product for 1 month before this study
10. *Blood donation*: blood donations for 90 calendar days before this study
11. *Drug, alcohol, caffeine, tobacco*: history of drug, alcohol [>1 drink/day for females and >2 drinks/day for males, defined according to the USDA Dietary Guidelines 2015-2020] caffeine (>5 cups coffee/tea/day) or tobacco abuse (≥10 cigarettes/day)
12. *Abuse drug test*: positive urine abuse drug test at screening or day -1
13. *Alcohol test*: positive alcohol breath test at day -1
14. *Diet*: abnormal diets (<1600 or >3500 kcal/day) or substantial changes in eating habits in the 4 weeks before this study; vegetarians; consumption of alcohol, grapefruit, products containing grapefruit, or beverages containing xanthines (coffee, tea, soda, coffee with milk, energy drinks) within 48 hours prior to the enrolment
15. Pregnancy (females only): positive or missing pregnancy test at screening or day -1, pregnant or lactating women
16. Vaccination within 4 weeks of study treatment
17. Other unspecified reasons that, in the opinion of the investigator, make the subject unsuitable for enrolment.

10.3.1 Not allowed treatments

No medication, including OTC, traditional Chinese medicine and herbal remedies, will be allowed for 2 weeks before the start of the study and during the whole study duration. Paracetamol will be allowed as therapeutic counter-measure for adverse events (AEs) according to the investigator's opinion. Hormonal contraceptives will be allowed.

The intake of any other medication will be reported as a protocol deviation.

11 STUDY SCHEDULE

The schedule of the study is summarised at page 11.

11.1 Study visits and procedures

Each study subject will undergo 6 visits.

The study protocol foresees a screening visit and a confinement lasting from Visit 2 to the Final Visit/early termination visit (ETV), i.e. from Day -1 to Day 8. Maximum study duration will be 22 days, screening visit included. A written informed consent will be obtained before any study assessment or procedure.

The first subject first visit (FSFV) is defined as the 1st visit performed at the clinical centre on the 1st screened subject. The last subject last visit (LSLV) is defined as the last visit performed at the clinical centre (or the telephonic follow-up, if applicable) on the last subject, i.e. the last visit foreseen by the study protocol, independently of the fact that the subject is a completer or a withdrawn subject.

The following phases, visits and procedures will be performed:

- **Screening phase**
  - Screening – visit 1: between day -14 and day -2
  - Visit 2: day -1

- **Interventional phase**
  - Visit 3: days 1-3
  - Visit 4: days 4-5
  - Visit 5: days 6-7

- **Final phase**
  - Visit 6: day 8 - Final visit/ETV. In case of early discontinuation, discontinued subjects will undergo an ETV

Activities to be performed are listed by visit in the following table:

Table 11.1.1 Schedule of study activities and procedures

	Day	Procedures/Assessments	Notes
Screening – Visit 1	*From day -14 to day -2*	➢ Explanation to the subject of study aims, procedures and possible risks ➢ Informed consent signature ➢ Screening number (e.g. [redacted], etc.) ➢ Demographic data and life style recording ➢ Medical/surgical history ➢ Previous/concomitant medications ➢ Full physical examination (body weight, height, vital signs, physical abnormalities) ➢ ECG recording ➢ Clinical laboratory analyses: haematology, blood chemistry, urinalysis, serum virology, bacteriology and pregnancy test (women) ➢ Urine multi-drug kit test ➢ AE monitoring ➢ Inclusion/exclusion criteria evaluation ➢ Eligibility evaluation	The subject screening numbers (e.g. [redacted], etc.) will be used to identify the subjects
Visit 2	*Day -1*	➢ Alcohol breath test ➢ Urine multi-drug kit test ➢ Urine pregnancy test (women) ➢ Inclusion/exclusion criteria evaluation ➢ Eligibility evaluation ➢ Enrolment ➢ Study subject number will be the same as the screening number that was initially assigned when the subject is successfully enrolled (e.g screening number .) ➢ Check of AEs and concomitant medications	Arrival at the clinic before the evening (before 18:30) Confinement until the morning of day 8 Standardised dinner Fasting for at least 10 h (overnight) before first dosing
Visit 3	*Day 1*	➢ Vital sign measurement at pre-dose ➢ IMP administration at 08:00 ± 1 h; intravenous infusion for 5 min ➢ Vital signs measurement at 1 h post-dose ➢ Blood sample collection for pharmacokinetic analysis at: pre-dose (0) and 5 (at the end of the infusion), 8, 12, 15, 20, 25, 30, 60 min, 2, 4, 6, 8, 10 and 12 h post-dose ➢ Urine sample collection for pharmacokinetic analysis at: 0-4; 4-8; 8-12 and 12-24 h post-dose ➢ Check of AEs and concomitant medications	Standardized lunch at 13:00 standardized dinner at 21:00
	Day 2	➢ Blood sample collection for pharmacokinetic analysis at 24 and 32 h post-dose ➢ Urine sample collection for pharmacokinetic analysis at 24-32 h post-dose ➢ Check of AEs and concomitant medications	Standardized breakfast around 9:00 Standardized lunch around 13:00 Standardized dinner around 21:00
	Day 3	➢ Vital sign measurement at 48 h post-dose ➢ Physical examination (body weight, physical abnormalities) at 48 h post-dose ➢ Clinical laboratory analyses: haematology, blood chemistry, urinalysis at 48 h post-dose ➢ Check of AEs and concomitant medications	Standardized breakfast around 9:00 Standardized lunch around 13:00 Standardized dinner around 21:00

	Day	Procedures/Assessments	Notes
Visit 4	*From day 4 to day 5*	➢ IMP administration at 08:00 ± 1 h; infusion for 5 min ➢ IMP administration at 20:00 ± 1 h; infusion for 5 min ➢ Blood sample collection for pharmacokinetic analysis at: pre-dose (0) before the morning infusion ➢ Check of AEs and concomitant medications	Standardized breakfast around 9:00 Standardized lunch around 13:00 Standardized dinner around 21:00
Visit 5	*Day 6*	➢ Vital sign measurement at pre-dose ➢ IMP administration at 08:00 ± 1 h; infusion for 5 min ➢ Vital signs measurement at 1 h post-dose ➢ Blood sample collection for pharmacokinetic analysis at: pre-dose (0) and 5 (at the end of the infusion), 8, 12, 15, 20, 25, 30, 60 min, 2, 4, 6, 8, 10 and 12 h post-dose ➢ Urine sample collection for pharmacokinetic analysis at: 0-4; 4-8; 8-12 and 12-24 h post-dose ➢ Check of AEs and concomitant medications	Standardized lunch at 13:00 (about 5 h post-dose) Standardized dinner at 21:00 (about 13 h post-dose)
	Day 7	➢ Blood sample collection for pharmacokinetic analysis at 24 and 32 h post-dose ➢ Urine sample collection for pharmacokinetic analysis at 24-32 h post-dose ➢ Check of AEs and concomitant medications	Standardized breakfast around 9:00 Standardized lunch around 13:00 Standardized dinner around 21:00
Final Visit/ETV	*Day 8 or early termination visit (ETV) in case of discontinuation*	➢ Vital sign measurement at 48 h post-dose (or at ETV) ➢ Physical examination (body weight, physical abnormalities) ➢ Clinical laboratory analyses: haematology, blood chemistry, urinalysis ➢ Check of AEs and concomitant medications In case of clinically significant results at the final visit, the subjects will be followed-up by the investigator until the normalisation of the concerned clinical parameter(s)	Discharge from the clinical centre in the morning of Day 8 after vital sign measurement, blood sampling for clinical laboratory assays and physical examination or in case of ETV. Upon leaving, the subjects will be instructed to contact immediately the investigator in case of occurrence of any untoward medical occurrence

11.2 Diet and lifestyle

The subjects will not take any food or drinks (except water) for at least 10 h (i.e. overnight) before the first drug administration (Day 1) and will remain fasted up to 5 h post-dose.
During the study, water will be allowed as desired. In order to maintain an adequate hydration, the subjects will be encouraged to drink at least 120 mL of mineral water every 2 h for 6 h post-dose on Days 1 and 6.

Coffee, tea or food containing xanthines (i.e. coffee, tea, soda, coffee with milk, energy drinks, etc.), alcohol and grapefruit will be forbidden starting 48 h before the enrolment until the end of the study. Smoking is not allowed for the whole study duration.

During confinement, routine ambulant daily activities will be strongly recommended.

The timing of each meal to be served during confinement is shown in Table11.1.1 above.
In the evening of day -1, upon confinement, the subjects will receive a light dinner before fasting overnight.
On days 1 and 6 (PK sampling collection days), standardised lunch and dinner will be served.
On days 2, 3, 4, 5 and 7, standardised breakfast, lunch and dinner will be served.

11.2.1 Restrictions

During the study, the subjects will be confined from the evening preceding the first drug administration (study day -1) until the morning of day 8 (after the final visit). They will attend the clinic in the evening of day -1 not later than 18:30.

During confinement, hazardous, strenuous or athletic activities will not be permitted.

12 DESCRIPTION OF SPECIFIC PROCEDURES

12.1 Physical examination

Full physical examinations will be performed at the screening visit, visit 3, day 3 and final visit/ ETV. Information about the physical examination will be recorded by the investigator. Any abnormalities will be recorded.

Significant findings/illnesses, reported after the start of the study and that meet the definition of an AE (see § 16), will be recorded in the subject source documents.

Date of the physical examination, overall investigator's interpretation (as normal or abnormal and, if abnormal, clinically significant or not clinically significant) and clinically significant abnormalities (if any) will be reported in the individual CRFs.

12.1.1 Body weight

Body weight will be recorded during each physical examination.
Subjects will be weighed (kg) lightly clothed without shoes. Height will be measured at screening only and BMI will be recorded. BMI will be calculated as weight [kg]/(height [m] x height [m]).

12.1.2 Vital signs

Subjects blood pressure (BP) and heart rate (HR) will be measured by the investigator or his/her deputy after 5 min at rest (in sitting position) at:

- Screening
- Visit 3, Day 1: at pre-dose and 1 h post-dose
- Visit 3, Day 3: at 48 h post-dose
- Visit 5, Day 6: at pre-dose and 1 h post-dose
- Final visit (at 48 h post-dose)/ETV

12.1.3 ECGs

One 12-lead ECG will be performed (in sitting/supine position) at screening only.

12.2 Clinical laboratory assays

Samples of blood and urine will be collected. The following laboratory analyses will be performed at the screening visit:

Haematology
Leukocytes and leukocyte differential count (percentage values and absolute values), erythrocytes, haemoglobin (conv. units), haemoglobin (IS units), haematocrit, MCV, MCH, MCHC, thrombocytes.

Blood chemistry
Electrolytes: sodium, potassium, calcium, chloride, inorganic phosphorus
Enzymes: alkaline phosphatase, γ-GT, AST, ALT
Substrates/metabolites: total bilirubin, creatinine, glucose, BUN or urea, uric acid, total cholesterol, triglycerides
Proteins: total proteins

Serum pregnancy test (women).

Urine analysis
Urine chemical analysis (stick)**:** pH, specific weight, appearance, colour, nitrites, proteins, glucose, urobilinogen, bilirubin, ketones, haematic pigments, leukocytes
Urine sediment (analysis performed only if positive): leukocytes, erythrocytes, flat cells, round cells, crystals, cylinders, mucus, bacteria

Serum virology and bacteriology
Hepatitis B (HBs antigen), **Hepatitis C** (HCV antibodies), **HIV 1/2** (HIV Ag/Ab combo), *Treponema pallidum*.

The same analyses, with the exception of virology, bacteriology and serum pregnancy test, will be performed also at Visit 3, Day 3 (48 h post-dose after the single dose) and at the final visit/ETV.

A urine drug test will be performed at the clinical centre at screening, using a urine multi-drug kit. The following drugs will be assessed: cocaine, amphetamine, methamphetamine, cannabinoids (delta-9-tetrahydrocannabinol - THC), opiates. The same test will be repeated upon confinement at Visit 2, day -1.

A serum pregnancy test will be performed by the laboratory at screening. A urine pregnancy test will be performed at Visit 2, day -1 at the clinical centre, upon confinement.

Date/time of samples collection, overall investigator's interpretation (as normal or abnormal and, if abnormal, clinically significant or not clinically significant) and clinically significant findings (if any) will be reported in the individual CRFs. All clinically significant abnormalities after the screening visit will be recorded as AEs. Hard copies of the laboratory print-outs will be attached to the CRFs.

12.3 Sampling for pharmacokinetic analysis

12.3.1 Venous blood sampling

Venous blood samples (up to 10 mL) will be collected from a forearm vein at the following times, both on Day 1, 2, after the single dose, and on Day 6, 7, after the last multiple dose:

- at pre-dose (0) and 5 (at the end of the infusion), 8, 12, 15, 20, 25, 30, 60 min, 2, 4, 6, 8, 10, 12, 24 and 32 h post-dose

The start of infusion (not the end) will be considered as time 0.

Actual sampling times for each subject will be recorded in the individual case report forms (CRFs). The actual sampling times should not exceed the recommended tolerance ranges presented in the following table. Any deviation outside the recommended ranges will be verified through Data Clarification Forms and, if confirmed, will be reported as protocol deviation, although it will not automatically lead to the exclusion of the concerned subjects from the PK analysis set.

Table 12.3.1.1 Tolerance ranges for the scheduled sampling times

Sampling time	Tolerance range
Pre-dose (0)	Within 30 minutes before IMP administration
0.0833 h (5 min), 0.1333 (8 min) 0.200 h (12 min), 0.250 h (15 min)	20 seconds
0.3333 h (20 min), 0.4167 (25 min), 0.5 h (30 min)	± 1 min
1 h	± 3 min
2, 4 h	± 5 min
6, 8, 10, 12, 24, 32 h	± 10 min

Blood samples for PK analysis will be collected using an indwelling catheter with switch valve. The cannula will be rinsed, after each sampling, with about 1 mL of sterile saline solution. The first mL of blood will be discarded at each collection time.

The remaining blood sample will be collected into blood collection tube.

Storage on ice will not last longer than 10 min until centrifugation at 4° C for 10 min at 2500xg to obtain plasma. Plasma will be collected into separate tubes, where an appropriate volume of DTT solution (50 µL DTT solution/mL plasma, i.e. 5% of the plasma volume according to the ratio 75 µL of DTT solution for 1.5 mL of plasma) will be added. The samples will be mixed shortly (Vortex, 20 sec), divided into two aliquots, P1 and P2 and transferred to ≤-70° C as soon as possible. The DTT solution (5 mg DTT/mL of water) will be freshly prepared in the evening before each sampling day and kept refrigerated until use. The preparation of the solution will be recorded in appropriate forms.

If any clinical assessment, such as vital signs measurement, is foreseen at the same time-point as blood sampling for PK analysis, blood collection will be performed at the scheduled time. However, vital signs can be influenced by the blood sampling. Therefore, these assessments can be performed within 30 min before the pre-dose PK time point (0 h) and within 10 min before the other scheduled PK time-points. Any deviations outside the recommended time will be verified through Data Clarification Forms. However, since vital signs measurements will be performed for safety reasons only, deviations from the planned time schedule will be considered not relevant.

12.3.2 Urine collection

Urine for PK will be collected in the following time intervals both on Day 1, 2, after the single dose, and on Day 6, 7 after the last multiple dose:

- 0-4; 4-8; 8-12, 12-24 and 24-32 h post-dose

Bladder must be emptied before the end of each collection period. During each interval, urine will be collected into containers, kept refrigerated at approximately 4° C and containing DTT solution. At the end of each collection interval, urine volume will be measured and after thorough mixing, two aliquots of 10 mL each (U1 and U2) will be prepared in polypropylene tubes. Urine volume collected in each collection interval will be accurately recorded in appropriate forms and in the CRFs. The two aliquots will be stored at ≤-70° C. The DTT solution (5 mg DTT/mL of water) will be freshly prepared in the evening before each sampling day and kept refrigerated until use. The preparation of the solution will be recorded in appropriate forms.

12.3.3 Analytics

The concentration of total NAC in plasma and urine will be determined at a certified bioanalytical laboratory to be designated in China, using a fully validated LC-MS/MS method, with a lower quantification limit (LQL) of 10 ng/mL.

Analyses will be performed according to the general Principles of "OECD Good Laboratory Practices for testing of chemicals" C(81) 30 (final) and GCP/ CFDA GCP (see also § 9.2).

The method validation report and the analytical report will be attached to the final report.

12.3.4 Labelling, storage and transport of samples

12.3.4.1 Samples labelling

Each sample tube will be clearly and unequivocally identified with a label resistant to the storage temperature and reporting:

Study code	Sponsor code Z7244J01
Subject number	Subject or Process or Unique Subject Identification number, e.g. CCI, etc.
Tube identification	CCI
Study day	1 or 6
Scheduled sampling time	as min and h; see § 12.3.1

12.3.4.2 Samples storage and transport

During the study the samples will be stored at ≤-70° C. At the end of each collection day, aliquots 1 and 2 will be stored in separate freezers.

All aliquots 1, packed in sufficient solid CO_2, will be shipped by an authorised courier from the clinical site, China, to Eurofins Central Laboratory, Shanghai, China. Aliquots 1 will remain stored at Eurofins Central Laboratory or a subcontracted bioanalytical laboratory to be designated until finalisation of the bioanalytical report. Afterwards, the samples will be destroyed and a certificate of destruction will be provided to the sponsor.

The counter-samples (aliquot 2) will remain stored at the clinical site, China. These samples could either be:

- sent to the laboratory for reanalysis should this become necessary for analytical reasons or if any problems occur during the delivery of aliquots 1, or
- destroyed at an authorised site, or
- transferred to the sponsor upon written request.

No analyses different from those stated in this protocol and agreed by the subjects when signing the informed consent form will be performed unless a new informed consent and a new approval from the Ethical Committee is obtained. The subjects may ask to destroy their own samples at any time.

13 ASSIGNMENT OF STUDY TREATMENT

13.1 Randomisation

No randomisation will take place in the present study. All the subjects will receive the same treatment.

13.2 Blinding

This is an open study. No masking procedure will be applied.

14 EVALUATION PARAMETERS

14.1 Study variables

14.1.1 Primary variables

After single dose:

- C_{max}, t_{max}, k_{el}, $t_{1/2}$, $AUC_{(0-t)}$, $AUC_{(0-\infty)}$, $AUC_{(0-12)}$, V_d, CL_t, $Ae_{(0-t)}$, $Fe_{(0-t)}$ and CL_r

After multiple doses:

- C_{ss_max}, t_{ss_max}, C_{ss_min}, $AUCss_{(0-t)}$, $AUCss_{(0-12)}$, C_{ss_av}, R, DF% and $Aess_{(0-t)}$.

14.1.2 Secondary variables

- TEAEs, vital signs (BP, HR), body weight, physical examinations, laboratory parameters.

14.2 Pharmacokinetic assessments

14.2.1 Pharmacokinetic parameters

The following PK parameters will be measured and/or calculated for plasma NAC, using the validated software Phoenix® WinNonlin® version 6.3 or higher (Certara USA, Inc., Princeton, NJ) (actual version will be stated in the final report), after single dose of IMP:

C_{max}:	Maximum NAC plasma concentration
t_{max}:	Time to achieve C_{max}
k_{el}:	Terminal elimination rate constant, calculated, if feasible, by log-linear regression using at least 3 points
$t_{1/2}$:	Half-life, calculated, if feasible, as $\ln 2/k_{el}$
$AUC_{(0-t)}$:	Area under the concentration-time curve from single dose to the last observed concentration time t, calculated with the linear up/log down trapezoidal method
$AUC_{(0-\infty)}$:	Area under the concentration-time curve extrapolated to infinity, calculated, if feasible, as $AUC_{(0-t)} + C_t/k_{el}$, where C_t is the last measurable drug concentration
$AUC_{(0-12)}$:	Area under the concentration-time curve at steady-state in the tau interval (from single dose to 12 h post-dose), calculated with the linear up/log down trapezoidal method

V_d: Volume of distribution associated with the terminal slope, calculated, if feasible, as Dose/($AUC_{(0-\infty)}$*k_{el})

CL_t: Total body clearance, calculated, if feasible, as Dose/ $AUC_{(0-\infty)}$

$Ae_{(0-t)}$: Total amount of NAC excreted in urine from single dose up to 32 h

$Fe_{(0-t)}$: Total fraction of NAC dose excreted in urine from single dose up to 32 h

CL_r: Renal clearance, calculated, if feasible, as $Ae_{(0-t)}$/ $AUC_{(0-\infty)}$

The following PK parameters will be measured and/or calculated for plasma NAC, using the same software, after multiple doses of IMP:

C_{ss_max}: Maximum NAC plasma concentration at steady-state

t_{ss_max}: Time to achieve C_{ss_max}

C_{ss_min}: Trough NAC plasma concentration at steady-state, measured as concentration at t=12 h

$AUCss_{(0-t)}$: Area under the concentration-time curve at steady-state from the last multiple dose to the last observed concentration time t, calculated with the linear up/log down trapezoidal method

$AUCss_{(0-12)}$: Area under the concentration-time curve at steady-state in the tau interval (from the last multiple dose to 12 h post-dose), calculated with the linear up/log down trapezoidal method

C_{ss_av}: Average NAC plasma concentration at steady-state, calculated as $AUCss_{(0-12)}$ /tau

R: Accumulation ratio, calculated as $AUCss_{(0-12)}$/ $AUC_{(0-12)}$

$Aess_{(0-t)}$: Total amount of NAC excreted in urine from the last multiple dose to 32 h at steady-state

DF%: Degree of fluctuation over one dosing interval at steady-state, calculated as (C_{ss_max} - C_{ss_min})/ C_{ss_av}*100

The sampling schedule is considered adequate if the ratio $AUC_{(0-t)}$/ $AUC_{(0-\infty)}$ equals or exceeds a factor of 0.8 (i.e. if $\%AUC_{extra}$ is <20%) for more than 80% of the individual PK profiles. This assures that the primary variable $AUC_{(0-t)}$ covers a sufficient percentage of the theoretical total extent of exposure.

The quality of log-linear regression (and, consequently, the reliability of the extrapolated PK parameters) should be demonstrated by a determination coefficient $R^2 \geq 0.8$. Individual extrapolated parameters, when considered unreliable, will be reported as NC (not calculated).

14.3 Safety assessments

Safety and general tolerability of the IMP will be based on TEAEs, physical examinations including body weight, vital signs and routine haematology, blood chemistry and urinalysis laboratory tests.

15 STATISTICAL METHODS

The data documented in this trial and the measured clinical parameters will be presented using classic descriptive statistics (i.e. total number of subjects treated [N], number of observations [n], mean standard deviation [SD], minimum [Min], median, maximum [Max]) for quantitative variables and frequencies (i.e. count and percentages) for qualitative variables if not stated otherwise.

Not available data will be evaluated as "missing values". The statistical analysis of demographic and safety data will be performed using the analysis software SAS® .

The statistical analysis of PK parameters will be performed using the validated software Phoenix® WinNonlin® version 6.3 or higher (Certara USA, Inc., Princeton, NJ) (actual version will be stated in the final report).

15.1 Analysis Sets

15.1.1 Definitions

A subject will be defined as screened after the signature of the informed consent, regardless of the completion of all the screening procedures.

A subject will be defined as eligible if he/she meets all the inclusion/exclusion criteria. Otherwise he/she will be defined as a screen failure.

A subject will be defined as enrolled in the study if he/she is included into the interventional phase of the study.

The following analysis sets are defined:

- Enrolled set: all enrolled subjects. This analysis set will be used for demographic, baseline and background characteristics
- Safety set: all subjects who receive at least one dose of IMP. This analysis set will be used for the safety analyses
- PK set: all enrolled subjects who fulfil the study protocol requirements in terms of IMP intake and have evaluable PK data readouts for the planned analyses, with no major deviations that may affect the PK results. This analysis set will be used for the statistical analysis of the PK parameters.

Each subject will be coded as valid or not valid for the safety and the PK set. Subjects will be evaluated according to the treatment they actually receive.

15.1.2 Reasons for exclusion from the PK set before bioanalysis

Reasons for the exclusion of subjects from the PK set are the following:

- intake of concomitant medications which could render the plasma concentration-time profile unreliable
- AEs which could render the plasma concentration-time profile unreliable
- administration errors which could render the plasma concentration-time profile unreliable
- other events which could render the plasma concentration-time profile unreliable

If one of these events occurs, it will be noted in the CRF during the study.

The samples from the subjects excluded from the PK set should still be assayed and the results listed. Subjects should not be excluded from the PK set if the AUC_{0-t} covers less than 80% of the $AUC_{0-\infty}$.

15.2 Sample size and power considerations

The sample size was not calculated through any statistical calculation. A sample size of 24 subjects was estimated as sufficient for the descriptive purposes of the present study.
Drop-out subjects will not be replaced.

15.3 Demographic, baseline and background characteristics

Critical demographic characteristics will be examined according to qualitative or quantitative data. Qualitative data will be summarised in contingency tables. Quantitative data will be summarised using classic descriptive statistics.

15.4 Analysis of pharmacokinetic parameters

15.4.1 Descriptive pharmacokinetics

A descriptive PK will be presented. The results will be displayed and summarised in tables and figures. Individual and mean curves (+SD at sampling times), indicating inter-subject variability, will be plotted. Data below the lower quantification limit (BLQL) will be considered as 0 in the calculations and presented as BLQL in listings and tables. As a consequence of BLQL (i.e. 0) values, calculated geometric means (if requested) could be null. For this reason, in the presence of any null value, the geometric mean will be reported as not calculated (NC).

15.5 Safety and tolerability evaluation

- **AEs**

AEs will be coded by System Organ Class (SOC) and Preferred Term (PT), using the Medical Dictionary for Regulatory Activities (MedDRA).
AEs will be classified as pre-treatment AEs (PTAEs) and TEAEs, according to the period of occurrence, as follows:

- PTAEs: all AEs occurring before the first dose of IMP and not worsening after the first dose of IMP
- TEAEs: all AEs occurring or worsening after the first dose of IMP

Individual PTAEs and TEAEs will be listed in subject data listings. No summary table will be provided for PTAEs. TEAEs will be summarised by treatment and overall. The number and percentage of subjects with any TEAE and the number of TEAEs will be tabulated by SOC and PT, seriousness, relationship to treatment and severity.

- **Physical examination**

Date of the physical examination, overall investigator's interpretation (as normal or abnormal and, if abnormal, clinically significant or not clinically significant) and clinically significant abnormalities (if any) will be listed.

- **Laboratory data**

Date/time of samples collection, overall investigator's interpretation (as normal or abnormal and, if abnormal, clinically significant or not clinically significant) and clinically significant findings (if any) will be listed. All laboratory results will be listed and a table of all the abnormal values will be presented. The overall investigator's interpretation will be summarised using tables of frequency.
The toxicity grading of laboratory tests is determined based on the NCI CTCAE V4.03.

- **Vital signs**

Vital signs values will be listed and summarised by descriptive statistics.

- **Body weight**

Body weight values will be listed and summarised by descriptive statistics.

16 DEFINITION AND HANDLING OF AEs AND SAEs

16.1 Applicable SOPs

AEs definition, classification and management will follow the CRO's SOPs, based upon applicable local and international regulations. The full SOPs or an operative summary will be made available to the clinical centre.

A brief summary of AE definition, classification and management is reported below.

16.2 Definition of Adverse Event (AE)

An Adverse Event is "*any untoward medical occurrence in a patient or clinical trial subject administered a medicinal product and which does not necessarily have a causal relationship with this treatment*".

AEs include:

- worsening (change in nature, severity or frequency) of conditions present at the onset of the trial
- patient/subject deterioration due to the primary illness
- intercurrent illnesses
- drug interactions
- events related or possibly related to concomitant medications
- abnormal laboratory values, as well as significant shifts from baseline within the range of normal, which the investigator considers to be clinically significant.

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

16.3 Definition of Adverse Drug Reaction (ADR)

An Adverse Reaction is "*any untoward and unintended response to an investigational medicinal product related to any dose administered*".
All AEs judged by either the reporting investigator or the sponsor as having a reasonable causal relationship to a medicinal product qualify as adverse reactions. The expression reasonable causal relationship means to convey in general that there is evidence or matter to suggest a causal relationship.

- **Unexpected Adverse Drug Reaction**

An unexpected ADR is: "*An adverse reaction, the nature, or severity of which is not consistent with the applicable product information (Reference Safety Information [RSI]).*

- **Reference Safety Information (RSI)**: in order to assess whether an adverse reaction is expected, the IB will be used.

16.4 Definition of Serious Adverse Events or Serious Adverse Drug Reaction

A Serious Adverse Event (SAE) is: "*any untoward medical occurrence or effect that at any dose*:

- results in death
- is life-threatening (i.e. the subject was at risk of death at the time of the event/reaction; it does not refer to an event/reaction which hypothetically might have caused death if it were more severe)
- requires inpatient hospitalization or prolongation of existing hospitalization
- results in persistent or significant disability/incapacity (where disability is defined as a permanent or substantial disruption of ability to carry out normal life functions, either reported or defined as per clinical judgement)
- is a congenital anomaly/birth defect
- is an important medical event that may not result in death, be life-threatening, or require hospitalization but, according to appropriate medical judgment, it may jeopardize the subject and may require medical or surgical intervention to prevent any of the outcomes listed in the definition above. Examples of such events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, and blood dyscrasias or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse.

Any suspected transmission via a medicinal product of an infectious agent is also considered a serious adverse event.

A **Suspected Unexpected Serious Adverse Reaction** (SUSAR) is an ADR that is both unexpected (not consistent with the applicable product information, e.g. IB) and also meets the definition of a SAE.

A non-serious adverse event is any adverse event that does not meet the criteria listed above for a serious adverse event.

16.5 Definition of Severity of Adverse Events

The term "severe" is used to describe the intensity (severity) of a specific event:

- Mild: causing no limitation of usual activities; the subject may experience slight discomfort.
- Moderate: causing some limitation of usual activities; the subject may experience annoying discomfort.
- Severe: causing inability to carry out usual activities; the subject may experience intolerable discomfort or pain.

16.6 Definition of Adverse Event causality

Causality shall be determined according to the definition of ADR given in 16.3.

All AE judged by either the investigator or the sponsor as having a reasonable suspected causal relationship to an investigational medicinal product qualify as adverse reactions. The causality assessment given by the investigator should not be downgraded by the sponsor.

The following binary decision for causality will be used:

- Reasonable possibility that the IMP caused the event
- No reasonable possibility that the IMP caused the event

Features supportive of an association include:

- temporal plausibility
- pharmacological properties of the drug or of the substance class
- course of the adverse event after dechallenge and, if applicable, after rechallenge
- specific tests indicating involvement of the drug in the occurrence/worsening of the adverse event
- alternative explanations

16.7 Adverse Events recording

Each AE occurring to a subject, either spontaneously revealed by the subject or observed by the investigator, whether believed by the investigator to be related or unrelated to the IMP, must be recorded on the AE information page of the eCRF (for SAEs information must be recorded also on the AE information page of the eCRF).
The Investigator will also perform an evaluation with respect to seriousness and causality (relationship of any AE to IMP) of the AEs. and record it on the appropriate section of the eCRF and records it on the appropriate section of the eCRF.

16.8 AEs monitoring window

- Start of monitoring: from immediately after the signature of the informed consent
- End of monitoring: final study visit/ETV (observation window)

An AE occurring after the final visit/ETV and coming to knowledge of the investigator (e.g. during a follow-up assessment or by spontaneous reporting by study subjects) must be recorded only if it is an ADR, according to the investigator's judgment.

16.9 Adverse Events reporting

The official language for reporting is English. The investigator and clinical staff of the present study are familiar with English language.

The investigator must report to the CRO all AEs which occur during the study, regardless of their relationship to the IMP. Protocol specific AEs or laboratory abnormalities critical to safety evaluations are to be identified in the protocol and reported to the sponsor according to reporting requirements and within the time periods specified.

All AEs are recorded by the investigator on the AE information page of the eCRF.

In addition, SAE will have to be reported according to the following detailed procedure.

16.9.1 SAEs reporting

With the exception of those SAEs that are identified as not requiring immediate reporting in the protocol, the investigator must report the SAEs to the CRO immediately and no later than 24 hours from when he/she becomes aware of the SAE, by faxing the "Serious Adverse Event Form" (back up plan) or e-mailing as scanned attachment (backup plan) or by Electronic Data Capture to the Drug Safety Unit (DSU) personnel (preferred method), as stated in the "List of CRO/ personnel" in § 16.13 of this protocol.

The community standards of confidentiality must always be maintained and any relevant national legislation on data protection must be followed.

Another copy of the "Serious Adverse Event Form" will be retained by the Investigator for the Investigator's file.

If the investigator becomes aware of any SAE occurred to a subject within the follow-up window established in the protocol, he/she will report the SAE as above. The SAE will be also reported in the eCRF.

If outside the follow-up window established in the protocol the investigator becomes aware of a SAE, if the investigator judges that the SAE is related to the study drug, it should be reported to the Sponsor. The Investigator might use the "Serious Adverse Event Form" via email or fax, but the SAE must not to be reported in the eCRF, as it is not an event occurred within the study period.

The investigator must report all SAEs that occur to the subjects to National Medical Products Administration/Local Ethics Committee/Provincial Food and Drug Administration/National Health and Family Planning Commission immediately no later than 24hours from when he/she becomes aware of SAE. Any SAEs that happen to the subjects outside the follow-up window should be reported by investigator to National Medical Products Administration/Local Ethics Committee/Provincial Food and Drug Administration/National Health and Family Planning Commission immediately.

16.10 Follow-up for Adverse Events

A follow-up "Serious Adverse Event Form" will be filled in if important follow-up information (i.e. diagnosis, outcome, causality assessment, results of specific investigations) is made available after submission of the initial AE Form for immediate reporting. Follow-up "Serious Adverse Event Form" will be reported to the Sponsor as above-described, under Section 16.9.1.

In any case of an AE that, in the opinion of the investigator, requires the subject's discontinuation, follow-up information relating to the subject's subsequent course must be collected until the event has subsided or the condition stabilised or when the subject is lost to follow up or death.

When follow-up data on non-serious AE are collected, information should be reported under "Comments" in the Final report of the CRF.

16.11 SUSARs management

The clock for initial expedited reporting starts as soon as the information containing the minimum reporting criteria has been received by the sponsor (day 0).
For fatal and life-threatening SUSARs the EC and Competent Authority (CA) should be informed as soon as possible and in any case within 7 days.
If the initial report is incomplete, e.g. not all the information/assessments were available, a complete report should be sent within an additional 8 days. Complete follow-up report should be sent within 15 calendar days.
SUSARs which are not fatal and not life-threatening are to be reported within 15 days.
The minimum information to be reported includes:

- Sponsor study number
- One identifiable coded subject
- One identifiable reporter
- One SUSAR
- One suspect IMP (including active substance name, code)
- A causality assessment (a reasonable possibility of a causal relationship with the study drug can be excluded only if there is information supporting this decision, otherwise it cannot be excluded).

16.12 Other events qualified for expedited reporting

Other safety issues also qualify for expedited reporting when they might materially alter the current benefit-risk assessment of a medicinal product or would be sufficient to consider changes in the medicinal product administration or in the overall conduct of the trial, for instance:

- single case reports of an expected serious adverse reaction with an unexpected outcome (e.g. a fatal outcome)
- an increase in the rate of occurrence of an expected serious adverse reaction, which is judged to be clinically important.
- post-study SUSARs that occur after the subject has completed a clinical trial and are reported to the investigator by the subject.
- new events relating to the conduct of the trial or the development of the medicinal product likely to affect the safety of the subjects, such as :
  - a SAE which could be associated with the trial procedures and which could modify the conduct of the trial
  - a significant hazard to the subject population such as lack of efficacy of a medicinal product used for the treatment of a life-threatening disease
  - a major safety finding from a newly completed animal study (such as carcinogenicity) or from other clinical trials.

16.13 SAEs: contacts

The investigator will report any SAE to the CRO. The CRO's details for SAEs are the following:
Email: [redacted]

The sponsor's details for SAEs are the following:
Email: [redacted]

16.14 Pregnancy

Subjects must be instructed that known or suspected pregnancy occurring during the study should be confirmed and reported to the Investigator, who must then withdraw the subject from the study without delay. The Investigator should also be notified of pregnancy occurring during the study but confirmed after its completion. In the event that a subject is subsequently found to be pregnant after inclusion in the study, then any pregnancy will be followed to the end of pregnancy or pregnancy termination and the status of mother and child will be reported to the Sponsor after delivery through the Pregnancy Report Form provided by the Sponsor. The Investigator will send pregnancy reports to DSU within the timeframes of SAEs, with follow-up information to be actively sought for the outcome of pregnancy.

- Part I of the Form is completed at the time of awareness of foetal exposure during pregnancy.
- Part II of the Form is filled in when information on pregnancy outcome becomes available.

If pregnancy results in abnormal outcome (spontaneous miscarriage, stillbirth and congenital anomalies) that the investigator considers to be due to the IMP, this will be treated as an expedited ADR report.

17 DATA MANAGEMENT PROCEDURES

17.1 Data collection – CRFs

The investigator must ensure that the clinical data required by the study protocol are carefully reported in the eCRFs. He/she must also check that the data reported in the eCRFs correspond to those in the subject's source documents.
ECG and laboratory results must be printed and signed by the Investigator and kept as source data on site after entering outcome into the eCRF.
All other data has to be documented in the subject file as source data first and then entered into the eCRF.
Data must be entered into eCRFs in English by the designated site personnel as soon as possible after a subject visit, and monitors will have access to data recorded. These data will be reviewed versus source documents by trial monitors for completeness and acceptability during monitoring visits.
If data correction is required for an eCRF after initial entry, the site personnel can make necessary corrections on their own or as a response to an automated query by the eCRF system. Monitor and Clinical Data Manager review the eCRF for accuracy and can also generate queries to the investigational staff for resolution.
Any correction to the eCRFs' entries must be carried out by the Investigator or a designated member of staff. Corrections are recorded in an audit trail that records the old information, the new information, and identification of the person making the changes, date of correction made and reason for change. In the interests of completeness of data acquisition, the questions which are repeated in each section of the eCRFs should be answered in full, even if there are no changes from a previous examination. A reasonable explanation must be given by the Investigator for all missing data. The Investigator or his/her designees named in the clinical staff list will review the eCRF for accuracy and completeness. The Investigator must electronically sign and date the eCRF pages as indicated.

17.2 Database management

The CRO will update and verify the database and create the final SAS data sets. The final data file will be transferred to the sponsor in the agreed format with all the other study documentation.

17.2.1 Coding dictionaries

Medical/surgical history and underlying diseases, clinically significant physical examination abnormalities and AEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA™).
Previous and concomitant medications will be coded using the WHO Drug Dictionary Enhanced (WHODDE). The version of the coding dictionaries will be stated in the study report.

18 STUDY MONITORING, QUALITY CONTROL AND QUALITY ASSURANCE

18.1 Monitoring

The monitoring visits will be conducted by appropriate staff of PAREXEL China Co. Ltd according to PAREXEL SOPs.

Monitoring activities, including monitoring purpose, selection and qualifications of monitors, extent and nature of monitoring, monitoring procedures, monitoring reports will comply with ICH-GCP chapter 5.18, CFDA GCP requirements and, when appropriate, further national regulatory guidelines.

Adequate time and availability for monitoring activities should be ensured by the investigator and key study personnel.

Data verification is required and will be done by direct comparison with source documents, always giving due consideration to data protection and medical confidentiality. In this respect the investigator will assure support to the monitor at all times.

The investigator agrees, by written consent to this protocol, to fully co-operate with compliance checks by allowing authorised individuals to have access to all the study documentation. In addition to the monitoring activities performed by the study monitor, the sponsor could perform some quality control activities to verify the compliance with the study procedures, the ICH-GCP guidelines and CFDA GCP.

18.2 Quality Control and Quality Assurance

The CRO has implemented and maintains a Quality System that includes quality controls and audits at different study steps with written SOPs to ensure that the study is conducted in compliance with the protocol and all effective amendments, ICH-GCP, and the applicable regulatory requirement(s) and that data have been reliably and correctly generated, recorded, processed and reported, in agreement with the ALCOAC principles (Attributable-Legible-Contemporaneous-Original-Accurate-Complete).

The clinical site is responsible for implementing and maintaining quality assurance and a quality control system to ensure that the study is conducted, and data are generated, documented (recorded), and reported in compliance with the protocol, ICH-GCP, CFDA GCP and any applicable regulatory requirement(s).
This protocol has been audited by the Sponsor QA.
The CRO and the sponsor will be responsible for their respective activities.

The sponsor may transfer any or all of the sponsor's trial-related duties and functions to a CRO, but the ultimate responsibility for the quality and integrity of the trial data always resides with the sponsor.

18.3 Applicable SOPs

The sponsor, the clinical centre and the CRO will follow their respective SOPs in the conduct of the respective activities, unless otherwise stated in written agreements. CRO/Sponsor's SOPs will be used for AE and SAE definition and management. SOPs will be made available for Sponsor's review, if required.

18.4 Data access

The investigator and the CRO will ensure that all source data, medical records, CRFs and all other documentation that is relevant to this study will be made accessible for monitoring activities, audits, IEC review, and regulatory inspections.

18.5 Audits and inspections

The sponsor, independent bodies acting on behalf of the sponsor and the CRO have the right to perform audits according to ICH-GCP, CFDA GCP and CFDA responsibilities.

The study may also be inspected by regulatory authorities.

The investigator and the CRO agree, by written consent to this protocol, to fully co-operate and support audits and inspections compliance checks by allowing authorised individuals to have access to all the study documentation.

19 ETHICAL CONSIDERATIONS

19.1 Ethics and Good Clinical Practice (GCP)

The study will be performed in accordance with the relevant guidelines of the Declaration of Helsinki.
The approval of the study protocol by the local IEC and by the Chinese Health Authorities will be obtained before the start of the study.

The present clinical study will be carried out according to the current revision of Good Clinical Practice (GCP), ICH topic E6 (R2), CFDA GCP and any applicable local law requirements.

19.2 Informed consent

Before being enrolled into the clinical study, the subjects must have expressed their consent to participate, after the investigator has explained to them, clearly and in details, the scope, the procedures and the possible consequences of the clinical study. Information will be given in both oral and written form. The information sheet and informed consent form will be prepared in the local language by the CRO and must be approved by the EC and regulatory authorities. It will include all the elements required by law according to the ICH-GCP and CFDA GCP recommendations.
In addition to the standard requirements that physicians are currently obliged to observe when providing information, the following points must also be covered:

- a description of the aims of the study and how it will be organised
- the type of treatment (information on the IMP and treatment procedures, as applicable)
- any potential negative effects attributable to the study product or treatment
- the freedom to ask for further information at any time
- the subjects' right to withdraw from the clinical study at any time without giving reasons and without jeopardising their further course of medical treatment
- the existence of a subject insurance cover and obligations following from this cover

Adequate time and opportunity to satisfy questions will be given to the subjects and the time will be recorded.

The investigator will be supplied with an adequate number of blank informed consent forms to be used. The forms will be signed and dated by both the investigator and the subject.
A copy of the signed form will be given to the subject.

To ensure medical confidentiality and data protection, the signed informed consent forms will be stored in the investigator's study file according to the regulatory requirements (see § 20.3).
The investigator will allow inspection of the forms by authorised representatives of the sponsor, EC members and regulatory authorities. He/she will confirm, by signing and dating the forms, that informed consent has been obtained.

19.3 Insurance policy

An insurance cover has been issued in favour of the subjects participating in this clinical study. The insurance is in compliance with the local regulation and with the requirements of the Health Authorities.

19.4 Withdrawal of subjects

It will be documented whether or not each subject completed the clinical study. If, for a subject, study treatment or observations are discontinued, the primary reason for discontinuation will be recorded.

19.4.1 Primary reason for discontinuation

- **Adverse event**: Any (significant) adverse event that in the opinion of the investigator or concerned subject is not compatible with study continuation. For the definition of AE, please refer to § 16.2.
- **death**: the absence of life or state of being dead
- **lost to follow-up**: the loss or lack of continuation of a subject to follow-up
- **non-compliance with study drug**: an indication that a subject has not agreed with or followed the instructions related to the study medication
- **physician decision**: a position, opinion or judgment reached after consideration by a physician with reference to the subject
- **pregnancy**: pregnancy is the state or condition of having a developing embryo or fetus in the body (uterus), after union of an ovum and spermatozoon, during the period from conception to birth
- **protocol deviation**: an event or decision that stands in contrast to the guidelines set out by the protocol
- **study terminated by sponsor**: an indication that a clinical study was stopped by its sponsor
- **technical problems**: a problem with some technical aspect of a clinical study, usually related to an instrument
- **withdrawal by subject**: study discontinuation requested by a subject for whatever reason
- **other**: different than the ones previously specified

For any subject discontinuing the study, the investigator will:

- ask the subject to undergo, as far as possible, a final medical visit (ETV) to examine the subject's health conditions and perform the required blood sampling for the laboratory assays. This examination will verify that all values tested at screening have remained within a clinically acceptable range (i.e. not clinically significant changes compared to screening)
- arrange for alternative medical care of the withdrawn subject, if necessary
- record the subject decision about the use of collected biological samples

- report in the CRF date and time of the last dose administration, and date and primary reason of study discontinuation
- record in the CRF any follow-up, if the subject is withdrawn for an AE

Discontinued subjects will not be replaced.

19.5 Study termination

The study will be considered terminated at the date of the last visit of the last subject or upon completion of any follow-up procedure described in protocol. The investigator and the sponsor have the right to discontinue the study at any time for reasonable medical and/or administrative reasons. As far as possible, this should occur after mutual consultation. Reasons for discontinuation have to be documented appropriately.

20 ADMINISTRATIVE PROCEDURES

20.1 Material supplied to the clinical centre

Beside IMP, the following study material will be supplied to the clinical centre:

- final version of the study protocol
- CRF for each subject plus some spare copies
- copy of the investigator's brochure (IB) relative to the IMP
- informed consent forms

Moreover, before the start of the study, the investigator will be provided with the following documents: CFDA GCP, ICH guidelines, confidentiality agreement (if applicable), protocol amendments (if any), declaration of Helsinki, insurance statement, SAE forms, financial agreement (if applicable), confidential subject identification code list form, drug accountability forms, investigator and study staff list form.

20.2 Protocol amendments

In order to obtain interpretable results, neither the investigator nor the sponsor will alter the study conditions agreed upon and set out in this protocol. Amendments should be made by mutual agreement between the investigator and the sponsor. Any amendment must be set out in writing, giving the reasons, and being signed by all concerned parties. The amendment becomes then part of the protocol.

20.3 Study documentation and record keeping

The investigator should ensure the accuracy, completeness, legibility, and timeliness of the data reported to the sponsor in the CRFs and in all required reports.

The investigator must keep source documents for each subject in the study. All information on the CRFs must be traceable to these source documents, which are generally stored in the subject's medical file. The source documents should contain all demographic and medical information, including laboratory data, ECGs, etc., and the original signed informed consent forms.

Data reported on the CRF that are derived from source documents should be consistent with the source documents or the discrepancies should be explained.

The investigator and the sponsor should maintain the study documents as specified in the "Essential Documents for the Conduct of a Clinical Trial" chapter 8 of ICH-GCP and as required by CFDA GCP and the applicable regulatory requirement(s).

These are documents which individually and collectively permit evaluation of a study and the quality of the data produced and include groups of documents, generated before the study commences, during the clinical study, and after termination of the study and include but are not limited to, study protocol, amendments, submission and approval of EC, raw data of subjects including lab tests and ECG tracing, insurance contracts, certificate of analysis of the IMP(s),

drug accountability records, signed informed consent forms, confidential subjects identification code, CRFs, curricula vitae of the investigator and other participants in the study, study staff lists and responsibilities, monitoring reports and final study report.

The investigator and the sponsor should take measures to prevent accidental or premature destruction of these documents.

Study documents must be retained by the investigator and the sponsor as long as needed to comply with ICH-GCP, CFDA GCP, national and international regulations. By signing the protocol, the investigator and the sponsor agree to adhere to these requirements.

20.4 Study subjects' recruitment

Study participants will be recruited from the volunteers' database. The study site may also use the site management organisation to recruit the study participants.

In addition, they may also use recruitment posters in other departments within the hospital, community outside or newspapers to raise awareness of the study that is going on.

Other departments within the hospital will be involved to recommend study participants as well.

The clinical site has detailed SOPs on the recruitment process.

20.5 Confidentiality and data protection

By signing this protocol, the investigator and the CRO agree to keep all the information provided by the sponsor in strict confidentiality and to request the same confidentiality from his/her staff. Study documents provided by the sponsor (protocols, IB, CRFs and other materials) will be stored appropriately to ensure confidentiality. The information provided by the sponsor to the investigator and to the CRO cannot be disclosed to others without direct written authorisation from the sponsor, except for the extent necessary to obtain the informed consent from the subjects wishing to participate in the study.

Data on subjects collected in the CRFs during the study will be documented in an anonymous way. If, as an exception, for safety or regulatory reasons identification of a subject becomes necessary, the monitor, the sponsor and the investigator will be bound to keep this information confidential.

20.6 Publication policy

The sponsor agrees that the study results (including negative and inconclusive as well as positive results) can be made publicly available by the investigator publishing in peer reviewed journals, presenting results at scientific congresses and posting information and results on internet-based public registers and databases.
Study results will be communicated in full to the competent Health Authorities by the submission of a complete clinical study report.

As the sponsor agrees that the study results can be published by the investigator(s), the investigator agrees to submit any manuscript (abstract, publication, paper, etc.) to the sponsor before any public disclosure.
This will be done in order to ensure that clinical study results are reported in an objective, accurate and balanced manner. The sponsor reviews the proposed manuscripts, before submission, within a reasonable period of time (30-90 days in relation with the complexity of the work).

The investigator will also be provided by the sponsor with the clinical study report and the results of any additional analysis, tables, figures, etc. undertaken for the purposes of the article, in order to take responsibility for the content of the publication(s).
On an exceptional basis, the sponsor may temporarily delay registration of certain data elements (e.g. compound, name, outcome, measures, etc.) to seek necessary intellectual property protection. This is because early disclosure of such data could, in some circumstances, prevent or negatively impact patentability.

21 STUDY RESPONSIBLE PERSONS

21.1 Sponsor

Zambon S.p.A., via Lillo del Duca 10, I-20091 Bresso, Italy
Phone: +39.02.66.52.41
Fax: +39.02.66.50.14.92

Protocol Review Committee Chairman
PPD

Sponsor's representatives
PPD

21.2 Drug assay

Eurofins Central Laboratory Shanghai, 395 Jiang Chang West Road, 7th Floor, Shanghai, 200436 China

Analytical facility:
United-Power Pharma Tech Co., Ltd. (UP-Pharma)
2F, Tower B, No. 33 Science Park Road,
Changping District, Beijing, P.R.China
ZIP Code: 102206

21.3 Project management, data analysis & reporting, monitoring

PAREXEL International (IRL) Limited ("**PAREXEL**"), a corporation organized under the laws of Ireland with a registered address at 70 Sir John Rogerson's Quay, Dublin 2, Ireland.

22 REFERENCES

1. Ventresca GP, Cicchetti V, Ferrari V. Drugs in Bronchial Mucology. 1989. Cap. 4.1 Thiols 77-102. Edited by PC Braga and Allegra. Raven Press Ltd
2. Aruoma OI, Halliwell B, Hoey BM, Butler J. The antioxidant action of N-acetylcysteine: Its reaction with hydrogen peroxide, hydroxyl radical, superoxide and hypochlorous acid. Free Radical Biol Med 1988; 6:593-597
3. Ziment I. Acetylcysteine: a drug that is much more than a mucokinetic. Biomed & Pharmacother 1988; 42: 513-520
4. Borgstrom L, Kagedal B, Paulsen O. Pharmacokinetics of N-acetylcysteine in man. Eur J Clin Pharmacol 1986; 31: 217-222
5. Olsson B, Johansson M, Gabrielsson J, Bolme P. Pharmacokinetics and bioavailability of reduced and oxidized N-acetylcysteine. Eur J Clin Pharmacol 1988; 34: 77-82
6. Burgunder JM, Varriale A, Lauterburg BH. Effect of N-acetylcysteine on plasma cysteine and glutathione following paracetamol administration. Eur J Clin Pharmacol 1989; 36: 127-131
7. De Caro L, Ghizzi A, Costa R, Longo A, Ventresca GP, Lodola L. Pharmacokinetics and bioavailability of oral acetylcysteine in healthy volunteers. Arzneim-Forsh/Drug Res 1989; 39: 382-386
8. Borgstrom L, Kagedal B. Dose dependent pharmacokinetics of N-acetylcysteine after oral dosing to man. Biopharmaceutics & Drug Disposition 1990; 11: 131-136
9. Frascio M. Pharmacokinetic study after single dose (1, 2, and 4 g) of a new N-acetylcysteine (NAC) oral formulation. Zambon Group Internal Report, code ZPM/94/34
10. Crestani S. Open label, randomized, cross-over, pharmacokinetic study to assess the bioequivalence of two oral N-Acetylcysteine preparations: 600 mg film coated tablets versus 600 mg effervescent tablets. 2002 Report in Zambon Group's file
11. Rusca A. Study CRO-PK-14-286 – Sponsor code Z7169J02. Comparative bioavailability study of N-acetyl-cysteine (NAC) 600 mg uncoated tablets vs. NAC 600 mg film-coated tablets in healthy male and female volunteers. CROSS Research S.A., Switzerland 2014
12. Rusca A. Study CRO-PK-07-189 – Sponsor code 7169J01. Comparative bioavailability study of a new oral N-acetyl-cysteine (NAC) formulation vs. Fluimucil®, Zambon in healthy volunteers. CROSS Research S.A., Switzerland, 2008
13. Rusca A. Study CRO-PK-09-225 – Sponsor code 7203J01. Pilot bioavailability study of a new 1200 mg N-acetyl-cysteine (NAC) effervescent tablet formulation vs. Fluimucil® 600 mg effervescent tablet (Zambon S.p.A., Italy) in healthy male and female volunteers. CROSS Research S.A., Switzerland, 2010
14. Brown M, Bjorksten A, Medved I and M McKenna. Pharmacokinetics of intravenous N-acetylcysteine in men at rest and during exercise. Eur J Clin Pharmacol 2004;60:717-723
15. Jones AL, Jarvier D. Simpson D. Pharmacokinetics of N-acetylcysteine are altered in patients with chronic liver diseases. Aliment Pharmacol Ther. 1997; 787-791
16. Prescott LF, Donovan JW, Jarvie DR, Proudfoot AT. The disposition and kinetics of intravenous N-acetylcysteine in patients with paracetamol overdosage. Eur J Clin Pharmacol 1989; 37: 501-506
17. Pendyala L, Creaven PJ. Pharmacokinetic and pharmacodynamic studies of N-acetylcysteine, a potential chemopreventing agent during a phase I trial. Cancer Epidemiology, Biomarkers and Prevention 1995; 4: 245-251
18. Rodenstein D, De Coster A, Gazzaniga A. Pharmacokinetics of oral acetylcysteine: absorption, binding and metabolism in patients with respiratory disorders. Clinical Pharmacokinetics 1978; 3: 247-254
19. Pharmacokinetics of N-Acetylcysteine following repeated i.v. infusions to hemodialysed chronic renal failure patients. Internal Report 2002. Zambon Group's file

20. Sjodin K, Nilsson E, Hallemberg A, Tunek A. Metabolism of N-acetyl-l-cysteine Biochem. Pharmacol 38 1989; 22: 3981-3985
21. Locatelli C. Fluimucil 20% injectable (N-Acetylcysteine). Expert Report on the clinical documentation 1996. Zambon Group's file
22. N-acetyl-L-cysteine (NAC) intravenous Investigator's Brochure, edition number 1, 20 April 2018
23. Guidelines on GMP, published by the Commission in The rules governing medicinal products in the European Community, Volume 4
24. Guidance on the investigation of bioequivalence. CPMP/EWP/QWP/1401/98 Rev. 1/Corr **, 20 January 2010
25. U.S. Department of Health and Human Services and U.S. Department of Agriculture, Nutrition and your health: 2015-2020 Dietary Guidelines, Appendix 9

CLINICAL STUDY PROTOCOL

Sponsor code Z7244J01

Phase I study to evaluate pharmacokinetics, safety and tolerability of single and multiple i.v. doses of N-acetylcysteine (NAC) in Chinese healthy volunteers

Single and multiple dose, single centre, open-label, one-way, pharmacokinetics, safety and tolerability clinical trial

Test formulation:	NAC 300 mg/ 3 mL solution for injection, Zambon S.p.A., Italy.
Sponsor:	Zambon S.p.A., via Lillo del Duca 10, I-20091 Bresso, Italy Phone: +39.02.66.52.41 Fax: +39.02.66.50.14.92
Investigator:	PPD - Principal investigator Ruijin Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, Phase I Unit, No.197, Rui Jin Er Road, Shanghai China Email PPD
Development phase:	I
Version and date:	Final version 3.0, 3 Dec 2019

This study will be conducted in accordance with the current version of Good Clinical Practice (GCP), ICH topic E6 (R2 and CFDA GCP)

1 PROTOCOL APPROVAL

1. SPONSOR

Zambon S.p.A., Italy

Sponsor's representative

PPD

PPD PPD

Date / / Signature

2. INVESTIGATOR

Principal investigator

I have read this protocol and agree to conduct this study in accordance with all the stipulations of the protocol and in accordance with the Declaration of Helsinki, the current revision of Good Clinical Practice (GCP), ICH topic E6 (R2), and the applicable local law requirements.

PPD

PPD

Date

Signature

PPD

2 STUDY SYNOPSIS

Title: Phase I study to evaluate pharmacokinetics, safety and tolerability of single and multiple i.v. doses of N-acetylcysteine (NAC) in Chinese healthy volunteers

Protocol number: Z7244J01

Clinical phase: Phase I

Study design: Single and multiple dose, single centre, open-label, one-way, pharmacokinetics, safety and tolerability clinical trial

Planned nr. of centres / countries: 1/China

Investigator and centre: Principal investigator: PPD, Ruijin Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, Phase I Unit, No.197, Rui Jin Er Road, Shanghai, China

Investigational medicinal product (IMP):
Test product: NAC, 300 mg/ 3 mL solution for injection, Zambon S.p.A., Italy

Dose regimen:
Two ampoules (300 + 300 mg) corresponding to a total dose of 600 mg of NAC diluted in 10 mL of NaCl 0.9% saline solution, will be administered by a 5-minute intravenous (i.v.) infusion.
Single dose:
One (1) dose of the investigational product will be administered under fasting conditions on day 1 at 08:00 ±1 h
Multiple dose regime:
Five (5) doses of the investigational product will be administered twice a day (b.i.d.) on days 4 and 5 at 08:00 ±1 h and 20:00 ±1 h and one dose will be administered on day 6 at 08:00 ±1.

Objective: To evaluate the NAC pharmacokinetics, safety and tolerability after single and multiple dose i.v. administration.

End-points:
Primary end-point:
- To evaluate pharmacokinetic parameters of NAC in plasma after single and multiple dose administration of the investigational product.

Secondary end-points:
- To collect safety and tolerability data after single and multiple dose administration of the investigational product.

Study variables:
Primary variables:
After single dose:
- C_{max}: maximum NAC plasma concentration
- t_{max}: time to achieve C_{max}
- k_{el}: terminal elimination rate constant, calculated, if feasible, by log-linear regression using at least 3 points
- $t_{1/2}$: NAC half-life, calculated, if feasible, as $ln2/k_{el}$
- $AUC_{(0-t)}$: area under the concentration-time curve from single dose to the last observed concentration time t, calculated with the linear up/log down trapezoidal method
- $AUC_{(0-\infty)}$: area under the concentration-time curve extrapolated to infinity, calculated, if feasible, as $AUC_{(0-t)} + C_t/k_{el}$, where C_t is the last measurable drug concentration
- $AUC_{(0-12)}$: area under the concentration-time curve at steady-state in the tau interval (from single dose to 12 h post-dose), calculated with the linear up/log down trapezoidal method
- V_d: volume of distribution associated with the terminal slope, calculated, if feasible, as $Dose/(AUC_{(0-\infty)}*k_{el})$
- CL_t: total body clearance, calculated, if feasible, as $Dose/ AUC_{(0-\infty)}$
- $Ae_{(0-t)}$: total amount of NAC excreted in urine from single dose up to 32 h
- $Fe_{(0-t)}$: total fraction of NAC dose excreted in urine from single dose up to 32 h
- CL_r: renal clearance, calculated, if feasible, as $Ae_{(0-t)}/ AUC_{(0-\infty)}$

STUDY SYNOPSIS (cont.)

Study variables (continued):
After multiple dose:

- C_{ss_max}: maximum NAC plasma concentration at steady-state
- t_{ss_max}: time to achieve C_{ss_max}
- C_{ss_min}: trough NAC plasma concentration at steady-state, measured as concentration at t=12 h
- $AUCss_{(0-t)}$: area under the concentration-time curve at steady-state from the last multiple dose to the last observed concentration time t, calculated with the linear up/log down trapezoidal method
- $AUCss_{(0-12)}$: area under the concentration-time curve at steady-state in the tau interval (from the last multiple dose to 12 h post-dose), calculated with the linear up/log down trapezoidal method
- C_{ss_av}: average NAC plasma concentration at steady-state, calculated as $AUCss_{(0-12)}$ /tau
- R: accumulation ratio, calculated as $AUCss_{(0-12)}$/ $AUC_{(0-12)}$
- DF%: degree of fluctuation over one dosing interval at steady-state, calculated as $(C_{ss\_max} - C_{ss\_min})/ C_{ss\_av}*100$
- $Aess_{(0-t)}$: total amount of NAC excreted in urine from the last multiple dose to 32 h at steady-state

Secondary variables:

- Treatment emergent adverse events (TEAEs), vital signs (blood pressure, heart rate), body weight, physical examinations, clinical laboratory parameters.

Analytics: total NAC will be determined in plasma and urine at a certified bioanalytical laboratory to be designated, using a LC-MS/MS validated method with a Lower Quantification Limit (LQL) of 10 ng/mL. Analytical facilities and procedures are in compliance with the general principles of GLP regulations

Sample size: Twenty-four (24) healthy male and female Chinese volunteers will be included in the study. Drop-out subjects will not be replaced.

Main selection criteria:
Inclusion criteria:

1. *Informed consent*: signed written informed consent before inclusion in the study
2. *Ethnicity, Sex and Age*: Chinese males and females, 18-45 year old inclusive
3. *Weight:* body weight ≥50 kg;
4. *Body Mass Index*: 19-26 kg/m^2 inclusive
5. *Vital signs*: systolic blood pressure 100-139 mmHg, diastolic blood pressure 50-89 mmHg, heart rate 50-90 bpm, measured after 5 min at rest in the sitting/supine position (to be chosen according to the usual procedure at the clinical site)
6. *Full comprehension*: ability to comprehend the full nature and purpose of the study, including possible risks and side effects; ability to co-operate with the investigator and to comply with the requirements of the entire study
7. *Nicotine addiction (smoker subjects only)*: ability to abstain from smoking for the duration of the clinical study
8. *Contraception and fertility (women only)*: women of child-bearing potential must be using at least one of the following reliable methods of contraception:
   a. Hormonal oral, implantable, transdermal, or injectable contraceptives for at least 60 calendar days before the screening visit
   b. A non-hormonal intrauterine device or female condom with spermicide or contraceptive sponge with spermicide or diaphragm with spermicide or cervical cap with spermicide for at least 60 calendar days before the screening visit
   c. A male sexual partner who agrees to use a male condom with spermicide
   d. A sterile sexual partner

   Women of non-child-bearing potential or in post-menopausal status for at least 1 year will be admitted.
   Women of childbearing potential should be willing to adopt abstinence or contraception measures during the study and two weeks post-dose.
   For all women, pregnancy test result must be negative at screening and day -1.

STUDY SYNOPSIS (cont.)

Exclusion criteria:

1. *Electrocardiogram (12-lead ECG in supine position)*: clinically significant abnormalities
2. *Physical findings*: clinically significant abnormal physical findings which could interfere with the objectives of the study
3. *Laboratory analyses*: clinically significant abnormal laboratory values indicative of physical illness, in particular significant laboratory abnormality indicative of hepatic condition (more than 3 times the upper limit)
4. *Allergy*: ascertained or presumptive hypersensitivity to the active principle and/or formulations' ingredients; history of anaphylaxis to drugs or allergic reactions in general, which the investigator considers may affect the outcome of the study
5. *Diseases*: significant history of renal, hepatic, gastrointestinal, cardiovascular, respiratory, skin, haematological, endocrine, urologic, metabolic, neurological or psychiatric diseases, as determined by the investigator, that may interfere with the aim of the study; history of carcinoma *in situ* and malignant disease; active bacterial or viral infection and fever >38° C within 48 h prior to study treatment administration
6. *Virology*: positive result of HIV, hepatitis B (HBV), hepatitis C (HCV) or *Treponema pallidum* (TP) assays
7. *Surgery*: any surgery within 2 months of screening (excluding diagnostic surgery)
8. *Medications*: medications, including over the counter (OTC) medications, herbal remedies and traditional Chinese remedies for 2 weeks before the start of the study. Hormonal contraceptives for women will be allowed
9. *Investigative drug studies*: participation in the evaluation of any investigational product for 30 calendar days before this study
10. *Blood donation*: blood donations for 90 calendar days before this study
11. *Drug, alcohol, caffeine, tobacco*: history of drug, alcohol [>1 drink/day for females and >2 drinks/day for males, defined according to the USDA Dietary Guidelines 2015-2020] caffeine (>5 cups coffee/tea/day) or tobacco abuse (≥10 cigarettes/day)
12. *Abuse drug test*: positive urine abuse drug test at screening or day -1
13. *Alcohol test*: positive alcohol breath test at day -1
14. *Diet*: abnormal diets (<1600 or >3500 kcal/day) or substantial changes in eating habits in the 4 weeks before this study; vegetarians; consumption of alcohol, grapefruit, products containing grapefruit, or beverages containing xanthines (coffee, tea, soda, coffee with milk, energy drinks) within 48 hours prior to the enrolment
15. Pregnancy (females only): positive or missing pregnancy test at screening or day -1, pregnant or lactating women
16. Vaccination within 4 weeks of study treatment
17. Other unspecified reasons that, in the opinion of the investigator, make the subject unsuitable for enrolment.

Schedule:

	Day	Procedures/Assessments	Notes
Screening – Visit 1	*From day -14 to day -2*	➢ Explanation to the subject of study aims, procedures and possible risks ➢ Informed consent signature ➢ Screening number (e.g. [redacted], etc.) ➢ Demographic data and life style recording ➢ Medical/surgical history ➢ Previous/concomitant medications ➢ Full physical examination (body weight, height, vital signs, physical abnormalities) ➢ ECG recording ➢ Clinical laboratory analyses: haematology, blood chemistry, urinalysis, serum virology, bacteriology and pregnancy test (women) ➢ Urine multi-drug kit test ➢ Adverse event (AE) monitoring ➢ Inclusion/exclusion criteria evaluation ➢ Eligibility evaluation	The subject screening numbers will be in running order (e.g. [redacted], etc.).

STUDY SYNOPSIS (cont.)

Schedule (continued):			
	Day	**Procedures/Assessments**	**Notes**
Visit 2	*Day -1*	➢ Alcohol breath test ➢ Urine multi-drug kit test ➢ Urine pregnancy test (women) ➢ Inclusion/exclusion criteria evaluation ➢ Eligibility evaluation ➢ Enrolment ➢ Subject study number will be the screening number if successfully enrolled (e.g. [redacted], etc.) ➢ Check of AEs and concomitant medications	Arrival at the clinic before the evening (before 18:30) Confinement until the morning of day 8 Standardised dinner Fasting for at least 10 h (overnight) before first dosing
Visit 3	*Day 1*	➢ Vital sign measurement at pre-dose ➢ IMP administration at 08:00 ± 1 h; intravenous infusion for 5 min ➢ Vital signs measurement at 1 h post-dose ➢ Blood sample collection for pharmacokinetic analysis at: pre-dose (0) and 5 (at the end of the infusion), 8, 12, 15, 20, 25, 30, 60 min, 2, 4, 6, 8, 10 and 12 h post-dose ➢ Urine sample collection for pharmacokinetic analysis at: 0-4; 4-8; 8-12 and 12-24 h post-dose ➢ Check of AEs and concomitant medications	Standardized lunch at 13:00 standardized dinner at 21:00
	Day 2	➢ Blood sample collection for pharmacokinetic analysis at 24 and 32 h post-dose ➢ Urine sample collection for pharmacokinetic analysis at 24-32 h post-dose ➢ Check of AEs and concomitant medications	Standardized breakfast around 9:00 Standardized lunch around 13:00 Standardized dinner around 21:00
	Day 3	➢ Vital sign measurement at 48 h post-dose ➢ Physical examination (body weight, physical abnormalities) at 48 h post-dose ➢ Clinical laboratory analyses: haematology, blood chemistry, urinalysis at 48 h post-dose ➢ Check of AEs and concomitant medications	Standardized breakfast around 9:00 Standardized lunch around 13:00 Standardized dinner around 21:00
Visit 4	*From day 4 to day 5*	➢ IMP administration at 08:00 ± 1 h; infusion for 5 min ➢ IMP administration at 20:00 ± 1 h; infusion for 5 min ➢ Blood sample collection for pharmacokinetic analysis at: pre-dose (0) before the morning infusion ➢ Check of AEs and concomitant medications	Standardized breakfast around 9:00 Standardized lunch around 13:00 Standardized dinner around 21:00

STUDY SYNOPSIS (cont.)

Schedule (continued):

	Day	Procedures/Assessments	Notes
Visit 5	*Day 6*	➢ Vital sign measurement at pre-dose ➢ IMP administration at 08:00 ± 1 h; infusion for 5 min ➢ Vital signs measurement at 1 h post-dose ➢ Blood sample collection for pharmacokinetic analysis at: pre-dose (0) and 5 (at the end of the infusion), 8, 12, 15, 20, 25, 30, 60 min, 2, 4, 6, 8, 10 and 12 h post-dose ➢ Urine sample collection for pharmacokinetic analysis at: 0-4; 4-8; 8-12 and 12-24 h post-dose ➢ Check of AEs and concomitant medications	Standardized lunch at 13:00 (about 5 h post-dose) Standardized dinner at 21:00 (about 13 h post-dose)
	Day 7	➢ Blood sample collection for pharmacokinetic analysis at 24 and 32 h post-dose ➢ Urine sample collection for pharmacokinetic analysis at 24-32 h post-dose ➢ Check of AEs and concomitant medications	Standardized breakfast around 9:00 Standardized lunch around 13:00 Standardized dinner around 21:00
Final Visit/ETV	*Day 8 or early termination visit (ETV) in case of discontinuation*	➢ Vital sign measurement at 48 h post-dose (or at ETV) ➢ Physical examination (body weight, physical abnormalities) ➢ Clinical laboratory analyses: haematology, blood chemistry, urinalysis ➢ Check of AEs and concomitant medications In case of clinically significant results at the final visit, the subjects will be followed-up by the investigator until the normalisation of the concerned clinical parameter(s)	Discharge from the clinical centre in the morning of Day 8 after vital sign measurement, blood sampling for clinical laboratory assays and physical examination or in case of ETV. Upon leaving, the subjects will be instructed to contact immediately the investigator in case of occurrence of any untoward medical occurrence

Life style and constraints:
During the study, the subjects will be confined from the evening preceding the first drug administration (study day -1) until the morning of day 8 (after the final visit).
The subjects will not take any food or drinks (except water) for at least 10 h (i.e. overnight) before the first drug administration (Day 1) and will remain fasted up to 5 h post-dose.
During the study, water will be allowed as desired. Coffee, tea or food containing xanthines (i.e. coffee, tea, soda, coffee with milk, energy drinks coke, chocolate, etc.), alcohol and grapefruit will be forbidden starting 48 h prior to the enrolment until the end of the study. Smoking is not allowed for the whole study duration.

STUDY SYNOPSIS (cont.)

Withdrawal of subjects:
It will be documented whether or not each subject completes the clinical study. In case of premature discontinuation of any subject, the primary reason for discontinuation will be recorded.

- Adverse event: Any (significant) adverse event that in the opinion of the investigator or concerned subject is not compatible with study continuation
- death: the absence of life or state of being dead
- lost to follow-up: the loss or lack of continuation of a subject up to follow-up
- non-compliance with study drug: an indication that a subject has not agreed with or followed the instructions related to the study medication
- physician decision: a position, opinion or judgment reached after consideration by a physician with reference to the subject
- pregnancy: pregnancy is the state or condition of having a developing embryo or fetus in the body (uterus), after union of an ovum and spermatozoon, during the period from conception to birth
- protocol deviation: an event or decision that stands in contrast to the guidelines set out by the protocol
- study terminated by sponsor: an indication that a clinical study was stopped by its sponsor
- technical problems: a problem with some technical aspect of a clinical study, usually related to an instrument
- withdrawal by subject: study discontinuation requested by a subject for whatever reason

For any subject discontinuing the study, the investigator will ask the subject to undergo, as far as possible, a final medical visit (ETV) to examine the subject's health conditions and perform the required blood sampling for the laboratory assays. This examination will verify that all values tested at screening have remained within a clinically acceptable range (i.e. not clinically significant changes compared to screening) and report in the case report form (CRF) date and time of the last dose administration, and date and primary reason of study discontinuation.

Data analysis:
The data documented in this trial and the measured clinical parameters will be presented using classic descriptive statistics (i.e. total number of subjects treated [N], number of observations [n], mean standard deviation [SD], minimum [Min], median, maximum [Max]) for quantitative variables and frequencies (i.e. count and percentages) for qualitative variables if not stated otherwise.

Analysis set:
Enrolled set: all enrolled subjects. This analysis set will be used for demographic, baseline and background characteristics
Safety set: all subjects who receive at least one dose of IMP. This analysis set will be used for the safety analyses
PK set: all enrolled subjects who fulfil the study protocol requirements in terms of IMP administration and have evaluable pharmacokinetic data readouts for the planned analysis, with no major deviations that may affect the pharmacokinetic results. This analysis set will be used for the statistical analysis of the pharmacokinetic results

Safety Assessments:
The statistical analysis of demographic and safety data will be performed using the analysis software SAS® .
The toxicity grading of laboratory tests is determined based on the NCI CTCAE V4.03. All AEs, adverse drug reactions and serious adverse events will be summarised by system organ class, severity and relationship to study drug.

Pharmacokinetics:
The statistical analysis of PK parameters will be performed using the validated software Phoenix® WinNonlin® version 6.3 or higher (Certara USA, Inc., Princeton, NJ) (actual version will be stated in the final report).
The individual plasma/urine concentration and pharmacokinetic parameters will be presented in listings and their descriptive statistics summarised in tables.

STUDY SYNOPSIS (cont.)

Background:
N-acetyl-L-cysteine (NAC) is the N-acetyl derivative of the naturally occurring amino acid L-cysteine.
NAC was introduced into the Italian pharmaceutical market in 1965 and it is now manufactured and marketed by Zambon S.p.A. in Europe, Asia, South America, Central America, and the Middle East, under various trade names (Ventresca 1989).
NAC is widely known as a mucolytic agent, with a well-established use in the treatment of respiratory tract disorders. However, after more than 35 years from its introduction in the clinical practice, this molecule continues to be the object of scientific interest due to its potential in the treatment of several conditions other than those related to the respiratory system. NAC can also act as a direct antioxidant agent due to the -SH group (Auroma 1988) and can easily penetrate into the cells where it is deacetylated to L-cysteine, thus supporting the biosynthesis of glutathione (Ziment 1988).
Clinical pharmacology and pharmacokinetics
Several pharmacokinetic (PK) studies were carried out in healthy volunteers (Borgstrom 1986, Olsson 1988, Burgunder 1989, De Caro 1989, Borgstrom 1990, Frascio, Crestani 2002, Rusca 2014, Rusca 2010, Rusca 2002). Further PK studies were carried out in patients with chronic liver damage (Jones 1997), with liver damage caused by paracetamol overdose (Prescott 1989), in subjects under long-term treatment for chemoprevention (Pendyala 1995), in patients with respiratory disorders (Rodenstein 1978) and in patients with End Stage Renal Diseases (Internal report 2002).
In the clinical studies, NAC was administered orally and intravenously both as single and repeated dose. Oral NAC was administered in different dosage forms, such as capsules, granulate, effervescent tablets, fast dissolving tablets, slow-release tablets.
Clinical trials and safety concerns
NAC has been available to patients as a mucolytic agent for many years and has a well-established and satisfactory safety and tolerability profile (see IB).
Independently of route of administration (inhalation, oral, intravenous) adverse events are reported rarely following NAC administration and are usually mild and transient.
The most frequent adverse events associated with NAC oral administration are gastrointestinal (e.g., vomiting, diarrhoea, stomatitis, abdominal pain and nausea). Hypersensitivity reactions (e.g. anaphylactoid reactions, urticaria, rash, pruritus, and bronchospasm) have been reported less frequently after oral administration, while with the IV administration they were reported more frequently compared to gastrointestinal symptoms. Some of the hypersensitivity reactions were associated to higher infusion rate. Most anaphylactoid reactions can be managed by temporarily suspending the acetylcysteine infusion, administering appropriate supportive care. A cumulative analysis of all safety reports received by the Sponsor up to September 2017 for NAC IV, confirmed the nature and severity of the events were in line with the above profile of the product.
Intravenous NAC has been studied in clinical trials and published case series both as mucolytic agent and as an antidote against intoxication by paracetamol and other toxic agents including *Amanita phalloides* and chlorinated hydrocarbons. Overall, in published studies in humans, a total of 745 patients were exposed to i.v. NAC at doses ranging from 500 mg/day to 980mg/kg/day for a duration of 1 to 18 days. Details of safety results are included in the IB.
In published studies, whereas i.v. NAC as mucolytic agent typically was given at doses up to 1000 mg/day, much higher i.v. doses of NAC as antidote were given over multiple days, maintaining a satisfactory safety profile.
Locatelli and colleagues published two case series, one of 86 patients with acute intoxication from *Amanita phalloides* and the other of 35 patients with acute intoxication from chlorinated hydrocarbons. Patients received i.v. NAC 300 mg/kg/day for 3 to 14 days and 3 to 18 days respectively. The safety and tolerability profile were overall benign, with 4 out of 121 exposed patients suffering from anaphylactoid reaction or rash (due to the rapid administration of the bolus according to the authors), none of which caused discontinuation of treatment. No other AEs were reported. (Locatelli 1996).

Rationale
NAC is widely known as a mucolytic agent, with a well-established use in the treatment of respiratory tract disorders. In the present study, the kinetic profile and the bioavailability of NAC will be investigated in Chinese healthy male and female volunteers all receiving the same single i.v. dose, i.e. a total dose of 600 mg of NAC, and the same multiple dose administrations, i.e. five i.v. doses of 600 mg of NAC administered b.i.d. for 2 days and once on the last day. The safety and tolerability will be monitored as well.
The present study will be part of the clinical development plan of NAC i.v. for registration in China of this formulation.

3 STUDY SCHEDULE

ACTIVITIES	Screening	Single Dose				Multiple Dose				Final visit/ETV[1]
Visit	V1	V2	V3			V4		V5		
	Day -14/-2	Day -1	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8[2]
Informed consent	x									
Demography and lifestyle	x									
Medical and surgical history	x									
Physical examination	x				x					x
Prior and concomitant medications	x	x	x	x	x	x	x	x	x	x
Height	x									
Body Weight	x				x					x
Clinical laboratory analysis (haematology, blood chemistry, urinalysis)	x				x					x
Virology and bacteriology	x									
Serum pregnancy test (women)	x									
Urine multi-drug kit test	x	x								
Blood pressure and heart rate	x		x[3]		x			x[3]		x[4]
Alcohol breath test		x								
Urine pregnancy test (women)		x								
ECG	X									
Inclusion/exclusion criteria	X	x								
Subject eligibility	X	x								
Enrolment		x								
Confinement		x	x	x	x	x	x	x	x	
Discharge										x
Investigational product administration			x[5]			x[6]	x[6]	x[5]		
Blood sampling			x[7]	x[7]		x[8]	x[8]	x[7]	x[7]	
Urine sampling			x[9]	x[9]				x[9]	x[9]	
Standardised meals		x[10]	x[11]	x[12]	x[12]	x[12]	x[12]	x[11]	x[12]	
Adverse event monitoring[13]	x	x	x	x	x	x	x	x	x	x

1. *Early termination visit (ETV)*
2. *Final visit on day 8 or in case of ETV*
3. *At pre-dose and 1 h post-dose*
4. *The vital signs check at 48 h post-dose (day 8), will correspond to the final measurement*
5. *At 8:00 ± 1 h: 5-min infusion*
6. *At 8:00 ± 1 h and at 20:00 ± 1 h; 5-min infusion*
7. *At pre-dose (0) and 5 (at the end of the infusion), 8, 12, 15, 20, 25, 30, 60 min and 2, 4, 6, 8, 10, 12, 24 and 32 h post-dose*
8. *At pre-dose (0)*
9. *0-4; 4-8; 8-12, 12-24 and 24-32 h post-dose*
10. *Standardised dinner*
11. *Standardised lunch and dinner*
12. *Standardised breakfast, lunch and dinner*
13. *Adverse events monitored starting at the screening visit, immediately after informed consent, up to the final visit/ETV*

4 TABLE OF CONTENTS

4.1 TABLES

Page

5 LIST OF ABBREVIATIONS

β-HCG	human chorionic gonadotropin β
γ-GT	γ-Glutamyl transpeptidase
ADR	Adverse Drug Reaction
AE	Adverse Event
ALT	Alanine aminotransferase
Ae_{0-t}	Total amount of NAC excreted in urine
$Aess_{(0-t)}$	Total amount of NAC excreted in urine at steady-state
ANOVA	Analysis of Variance
AST	Aspartate aminotransferase
BB	Blue book
BLQL	Below Lower Quantification Limit
BUN	Blood Urea Nitrogen
$AUC_{(0-t)}$	Area under the concentration-time curve from single dose to the last observed concentration time t
$AUC_{(0-\infty)}$	Area under the concentration-time curve extrapolated to infinity
$AUC_{(0-12)}$	Area under the concentration-time curve at steady-state in the tau interval
$AUCss_{(0-t)}$	Area under the concentration-time curve at steady-state from the last multiple dose to the last observed concentration time t
$AUCss_{(0-12)}$	Area under the concentration-time curve at steady-state in the tau interval
BMI	Body Mass Index
BP	Blood Pressure
CA	Competent Authority
CDISC	Clinical Data Interchange Standards Consortium
CFDA	China Food And Drug Administration
CI	Confidence Interval
C_{max}	Peak drug concentration
CLt	Total body clearance
CLr	Renal clearance
CPL	Clinical Project Leader
CRA	Clinical Research Associate
CRF	Case Report Form
CRO	Contract Research Organisation
CS	Clinically Significant
CSP	Clinical Study Protocol
CSR	Clinical Study Report
C_{ss_av}	Average NAC plasma concentration at steady-state
$C_{ss\ max}$	Maximum plasma concentration at steady-state
C_{ss_min}	Trough plasma concentration at steady-state
CV	Coefficient of Variation
DBP	Diastolic Blood Pressure
DF	Degree of fluctuation
DSU	Drug safety unit
DTT	Dithiothreitol
EC	Ethics Committee
ECG	Electrocardiogram
ETV	Early Termination Visit
FDA	Food and Drug Administration
Fe_{0-t}	Total fraction of NAC dose excreted in urine
F_{rel}	Relative Bioavailability
FSFV	First Subject First Visit
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
HBs Ag	Hepatitis B virus surface antigen
HCV Ab	Hepatitis C virus antibodies
HIV	Human Immunodeficiency Virus

HR	Heart Rate
IB	Investigator's Brochure
ICH	International Conference on Harmonisation
IRB/IEC	Institutional Review Board/Independent Ethics Committee
i.m.	Intramuscular
IMP	Investigational Medicinal Product
IUD	Intra-Uterine Device
i.v.	Intravenous
k_{el}	Terminal elimination rate constant
LQL	Lower Quantification Limit
LSLV	Last Subject Last Visit
MCH	Mean Cell Haemoglobin
MCHC	Mean Cell Haemoglobin Concentration
MCV	Mean Cell Volume
MedDRA	Medical Dictionary for Regulatory Activities
MRT	Mean Residence Time
MW	Molecular Weight
N	Normal
NA	Not Applicable
NAC	N-acetyl-L-cysteine
NC	Not calculated
NCS	Not clinically significant
NSAIDs	Non-Steroidal Anti-Inflammatory Drugs
OTC	Over The Counter
PE	Point Estimate
PK	Pharmacokinetics
PT	Preferred Term
PTAE	Pre-Treatment Adverse Event
R	Accumulation ratio
RBC	Red Blood Cells
RSI	Reference safety information
SAE	Serious Adverse Event
SBP	Systolic Blood Pressure
SD	Standard Deviation
SOC	System Organ Class
SOP	Standard Operating Procedure
SDTM	Study Data Tabulation Model
SUSAR	Suspected Unexpected Serious Adverse Reaction
T	Test
TEAE	Treatment-Emergent Adverse Event
THC	delta-9-tetrahydrocannabinol
$t_{1/2}$	Half-life
t_{max}	Time to achieve C_{max}
t_{ss_max}	Time to achieve C_{ss_max}
USDA	United States Department of Agriculture
Vd	Volume of distribution
WBC	White Blood Cells
WHODDE	World Health Organisation Drug Dictionary Enhanced

6 INTRODUCTION

6.1 Background

N-acetyl-L-cysteine (NAC) is the N-acetyl derivative of the naturally occurring amino acid L-cysteine.

NAC was introduced into the Italian pharmaceutical market in 1965 and it is now manufactured and marketed by Zambon S.p.A. in Europe, Asia, South America, Central America, and the Middle East, under various trade names (1).

NAC is widely known as a mucolytic agent, with a well-established use in the treatment of respiratory tract disorders. However, after more than 35 years from its introduction in the clinical practice, this molecule continues to be the object of scientific interest due to its potential in the treatment of several conditions other than those related to the respiratory system. NAC can also act as a direct antioxidant agent due to the -SH group (2) and can easily penetrate into the cells where it is deacetylated to L-cysteine, thus supporting the biosynthesis of glutathione (3).

6.2 Clinical pharmacology and pharmacokinetics

Several pharmacokinetic (PK) studies were carried out in healthy volunteers (4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14). Further PK studies were carried out in patients with chronic liver damage (15), with liver damage caused by paracetamol overdosage (16), in subjects under long-term treatment for chemoprevention (17), in patients with respiratory disorders (18) and in patients with End Stage Renal Diseases (19).
In the clinical studies, NAC was administered orally and intravenously both as single and repeated doses. Oral NAC was administered in different dosage forms, such as capsules, granulate, effervescent tablets, fast dissolving tablets, slow-release tablets.

In particular, Borgström *et al*. (4) studied for the first time the PK profile of NAC after i.v. dose of 600 mg of NAC infused over 5 min in 10 healthy male and female volunteers in comparison with 3 other oral dosage forms. The PK parameters obtained by the authors after i.v. infusion are summarised in the following table.

Table 6.2.1 PK parameters of plasma free NAC (N=10)

C_{max} (µmol/L)	t_{max} (h)	CL (L/kgxh)	CL_r (L/kgxh)	$t_{½}$ (h)	Ae_{0-12} (% of dose)
16.0±7.9	*0.65±0.33*	*0.207±0.017*	*0.058±0.011*	*2.27±0.32*	*29.0±3.2**

*mean±SD is reported; *: N=9; Source: 4*

However, the authors analysed NAC in deproteinised plasma thus missing the protein-bound NAC in their measurements.

Olsson *et al*. (5) improved the bioanalytical method and were able to measure the total NAC by reduction of the disulphide bonds in plasma before precipitating proteins. After single i.v. dose of 200 mg of NAC to 6 healthy volunteers, the authors found the following PK parameters:

Table 6.2.2 PK parameters of plasma total NAC (N=6)

	C_{max} (µM)	V_{ss} (L/kg)	CL (L/kgxh)
T	*121 (82.9 – 162)*	*0.47 (0.46 – 0.55)*	*0.11 (0.09 – 1.13)*

median (range) is reported; Source: 5

Jones *et al*. (15) studied the bioavailability of NAC after single i.v. dose of 600 mg of NAC infused over 3 min to patients with chronic liver disease, but also to 6 healthy male and female volunteers as control subjects. The authors found the following PK parameters for total NAC:

Table 6.2.3 PK parameters of plasma total NAC (N=6)

AUC (mg/Lxh)	CL_r (L/h)	$t_{½}$ (h)	Vd_{ss} (L)
93.9±9.6	*6.5±0.8*	*2.6±0.3*	*17.4±2.8*

mean±SD is reported; Source: 15

Brown *et al*. (14) investigated the PK of NAC infused i.v. to 24 healthy men at rest and during exercise. NAC was infused at the dose of 125 mg/kgxh for 15 min followed by 25 mg/kgxh for 35 min to healthy men at rest. Then, the infusion continued during exercise until fatigue. The authors found NAC peak concentrations in plasma at the end of the initial loading infusion. Mean peak concentration of total NAC was 205.1±68.3 mg/L, while mean CL was 0.164 L/kgxh.

It is worth noting that, while scientific literature shows that PK profiles of NAC after oral administration are very consistent (in terms of half-life, rate and extent of exposure, bioavailability, dose-linearity, etc.) among various publications, this is not the case for the data obtained after i.v. dosing. In fact, for this administration route, published studies differ in several key aspects, i.e., for example, type of administration, applied bioanalytical methods, drug doses, calculation methods for PK parameters. Furthermore, the number of subjects enrolled in each published study is very small. For these reasons, the comparability of the literature data is low among the published studies and potentially also to the expected results of the present study.

6.3 Clinical trials and safety concerns

NAC has been available to patients as a mucolytic agent for many years and has a well-established and satisfactory safety and tolerability profile (see IB).
Independently of route of administration (inhalation, oral, intravenous) adverse events are reported rarely following NAC administration and are usually mild and transient.
The most frequent adverse events associated with NAC oral administration are gastrointestinal (e.g., vomiting, diarrhoea, stomatitis, abdominal pain and nausea). Hypersensitivity reactions (e.g. anaphylactoid reactions, urticaria, rash, pruritus, and bronchospasm) have been reported less frequently after oral administration, while with the i.v. administration they were reported more frequently compared to gastrointestinal symptoms. Some of the hypersensitivity reactions were associated to higher infusion rate. Most anaphylactoid reactions can be managed by temporarily suspending the acetylcysteine infusion, administering appropriate supportive care.

A cumulative analysis of all safety reports received by the Sponsor up to September 2017 for NAC IV, confirmed the nature and severity of the events were in line with the above profile of the product.
Intravenous NAC has been studied in clinical trials and published case series both as mucolytic agent and as an antidote against intoxication by paracetamol and other toxic agents including *Amanita phalloides* and chlorinated hydrocarbons. Overall, in published studies in humans, a total of 745 patients were exposed to i.v. NAC at doses ranging from 500 mg/day to 980mg/kg/day for a duration of 1 to 18 days. Details of safety results are included in the IB.
In published studies, whereas i.v. NAC as mucolytic agent typically was given at doses up to 1000 mg/day, much higher i.v. doses of NAC as antidote were given over multiple days, maintaining a satisfactory safety profile.
Locatelli and colleagues published two case series, one of 86 patients with acute intoxication from *Amanita phalloides* and the other of 35 patients with acute intoxication from chlorinated hydrocarbons. Patients received i.v. NAC 300 mg/kg/day for 3 to 14 days and 3 to 18 days respectively. The safety and tolerability profile were overall benign, with 4 out of 121 exposed patients suffering from anaphylactoid reaction or rash (due to the rapid administration of the bolus according to the authors), none of which caused discontinuation of treatment. No other AEs were reported (21).

6.4 Rationale

NAC is widely known as a mucolytic agent, with a well-established use in the treatment of respiratory tract disorders.
In the present study, the kinetic profile and the bioavailability of NAC will be investigated in Chinese healthy male and female volunteers all receiving the same single i.v. dose, i.e. a total dose of 600 mg of NAC, and the same multiple dose administrations, i.e. five i.v. doses of 600 mg of NAC administered b.i.d. for 2 days and once on the last day. The safety and tolerability will be monitored as well.
The present study will be part of the clinical development plan of NAC i.v. for registration in China of this formulation.

6.5 Risk and benefits

NAC is a well-known drug which has been used for decades.

Undesired effects which may occur during treatment with NAC include: anaphylactic shock, anaphylactic reaction, anaphylactoid reaction, hypersensitivity, tachycardia, bronchospasm, dyspnoea, vomiting, nausea, angioedema, urticaria, flushing rash, pruritus, face oedema, blood pressure decreased, prothrombin time prolonged (for details refer to IB; 22).

Blood sampling with cannula insertion may cause minor discomfort. The risks associated with blood draws include pain, bleeding and bruising.

No specific benefits for the participants in the current study are foreseen. Their remuneration will be paid after study completion. The remuneration covers loss of time and any inconvenience caused by the participation in the study.

7 STUDY OBJECTIVES

The objective of the study is to evaluate the NAC pharmacokinetics, safety and tolerability after single and multiple dose i.v. administration.

7.1 Primary end-point

- To evaluate pharmacokinetic parameters of NAC in plasma after single and multiple dose administration of the investigational product.

7.2 Secondary end-point

- To collect safety and tolerability data after single and multiple dose administration of the investigational product.

8 CLINICAL SUPPLIES

8.1 Treatment

All the subjects enrolled in the study will receive the same treatment with the investigational medicinal product (IMP), i.e. NAC, 300 mg/ 3 mL solution for injection, as follows:

- on day 1 at 08:00 ±1 h, one dose of 600 mg of NAC (300 + 300 mg ampoule) will be administered under fasting conditions;

After a wash-out of 3 days:

- on days 4 and 5 at 08:00 ±1 h and 20:00 ±1 h and at 08:00 ±1 on day 6, 5 doses of 600 mg of NAC (300 + 300 mg ampoule) will be administered.

8.1.1 Description of products

8.1.1.1 Test product

IMP	NAC 300 mg/ 3 mL solution for injection
Active substance	N-acetyl-L-cysteine
Manufacturer (active substance)	F.I.S. Fabbrica Italiana Sintetici S.p.A., Via Dovaro, 36045 Lonigo (Vicenza), Italy (GMP compliant)
Manufacturer (finished product)	Zambon S.p.A., Via della Chimica 9, 36100 Vicenza, Italy (GMP compliant)
Pharmaceutical form	Solution for injection
Dose	300 mg/ 3 mL
Administration route	Parenteral

The analytical certificates will be supplied with the IMP. Quali-quantitative formulation is as follows:

Each 3 mL vial (10%) contains:
- N-acetyl-L-cysteine (NAC) 300 mg
- sodium hydroxide 74 mg
- disodium edetate 3 mg
- water for injections q.s. to 3 mL

8.1.2 Dose regimen

Two ampoules of IMP (300 + 300 mg) corresponding to a total dose of 600 mg of NAC diluted in 10 mL of NaCl 0.9% sterile saline solution, will be administered by a 5-minute i.v. infusion.

Single dose:

One (1) dose of IMP will be administered under fasting conditions on day 1 at 08:00 ±1 h.

Multiple dose regime:

Five (5) doses of IMP will be administered twice a day (b.i.d.) on days 4 and 5 at 08:00 ±1 h and 20:00 ±1 h and one dose will be administered on day 6 at 08:00 ±1.

8.1.3 Route and method of administration

Each dose of IMP will be prepared by diluting the content of 2 ampoules of IMP in 10 mL of saline, as described above (§ 8.1.2), and administered to each study subject at the clinical site only by the investigator or his/her deputy.

Each dose will be infused intravenously over 5 min.

The start of infusion (not the end) will be considered as time 0.

8.1.4 Investigational product distribution

The IMP will be exclusively used for the present clinical study and will only be administered to the subjects enrolled in the study.

8.2 Packaging and labelling

The IMP primary packaging will be glass vials.

The formulation labelling will report all the information requested according to the Annex 13 to the Good Manufacturing Practice (published by the Commission in The rules governing medicinal products in the European Community, Volume 4; 23) and in compliance with applicable laws and regulations as follows:

a. Name, address and telephone number of the sponsor, contract research organisation or investigator (the main contact for information on the product, clinical study and any emergency)
b. Pharmaceutical dosage form, route of administration, quantity of dosage units, and the name and strength
c. The batch and/or code number to identify the contents and packaging operation
d. A study reference code allowing identification of the study, site, investigator and sponsor if not given elsewhere

e. The study subject identification number/treatment number and where relevant, the visit number

f. The name of the investigator (if not included in (a) or (d))

g. Directions for use (reference may be made to a leaflet or other explanatory document intended for the study subject or person administering the product)

h. "For clinical study use only" or similar wording

i. The storage conditions

j. Period of use (use-by date, expiry date or re-test date as applicable), in month/year format and in a manner that avoids any ambiguity

k. "Keep out of reach of children"

Labels will be in local language.

8.3 Storage conditions

The IMP will be stored at ≤25° C in a dry locked place, sheltered from light.

8.4 Drug accountability

The IMP will be provided directly to the investigator by the Sponsor or designee, in excess of the amount necessary for the study (at least 25% excess).

After receipt of the IMP supply, the pharmacist will confirm in writing by signing and dating standard drug accountability forms.

At the end of the study, used, unused and partially used supplies of IMP provided by the Sponsor or designee will either be destroyed on site (upon written authorisation) or returned to the Sponsor or designee, after assessment of drug accountability.

9 INVESTIGATIONAL PLAN

9.1 Overall study design

This is a single and multiple dose, single centre, open-label, one-way, pharmacokinetics, safety and tolerability clinical trial of Phase I to be performed in Chinese healthy male and female volunteers.

9.2 Discussion of design

The study has been designed in agreement with the Chinese Technical Guideline on Clinical Pharmacokinetic Research of Chemical Drugs, 18 March 2005 and the European Guideline on the Investigation of Bioequivalence (CPMP/QWP/EWP/1401/98 Rev. 1, 20 January 2010) (24).

An open-label design is used since the primary end-point of the study is based on objective measurements of NAC in blood. The outcome variables are not influenced by the subjects or investigator being aware of the administered products.

Blood sampling time-points were selected on the basis of the known PK profile of NAC (4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14).

The dose of 600 mg has been selected since this is the efficacious, safe and well tolerated dose used in clinical practice (see also 22).

The bioanalysis will be performed in compliance with GCP and CFDA GCP regulations and in accordance with the applicable principles of GLP, as defined by OECD, in a GLP compliant facility. Moreover, sample analysis will be conducted in compliance with the Chinese Guidance on Bioanalysis: method validation and analysis of study samples (2015).

10 STUDY POPULATION

10.1 Target population

Chinese healthy male and female volunteers will be included in the study.

10.2 Inclusion criteria

To be enrolled in this study, subjects must fulfil all these criteria:

1. *Informed consent*: signed written informed consent before inclusion in the study
2. *Ethnicity, Sex and Age*: Chinese males and females, 18-45 year old inclusive
3. *Weight:* body weight ≥50 kg;
4. *Body Mass Index*: 19-26 kg/m^2 inclusive
5. *Vital signs*: systolic blood pressure 100-139 mmHg, diastolic blood pressure 50-89 mmHg, heart rate 50-90 bpm, measured after 5 min at rest in the sitting/supine position (to be chosen according to the usual procedure at the clinical site)
6. *Full comprehension*: ability to comprehend the full nature and purpose of the study, including possible risks and side effects; ability to co-operate with the investigator and to comply with the requirements of the entire study
7. *Nicotine addiction (smoker subjects only)*: ability to abstain from smoking for the duration of the clinical study
8. *Contraception and fertility (women only)*: women of child-bearing potential must be using at least one of the following reliable methods of contraception:
    a. Hormonal oral, implantable, transdermal, or injectable contraceptives for at least 60 calendar days before the screening visit
    b. A non-hormonal intrauterine device or female condom with spermicide or contraceptive sponge with spermicide or diaphragm with spermicide or cervical cap with spermicide for at least 60 calendar days before the screening visit
    c. A male sexual partner who agrees to use a male condom with spermicide
    d. A sterile sexual partner

    Women of non-child-bearing potential or in post-menopausal status for at least 1 year will be admitted.

    Women of childbearing potential should be willing to adopt abstinence or contraception measures during the study and two weeks post-dose.

    For all women, pregnancy test result must be negative at screening and day -1.

10.3 Exclusion criteria

Subjects meeting any of these criteria will not be enrolled in the study:

1. *Electrocardiogram (12-lead ECG in supine position)*: clinically significant abnormalities
2. *Physical findings*: clinically significant abnormal physical findings which could interfere with the objectives of the study
3. *Laboratory analyses*: clinically significant abnormal laboratory values indicative of physical illness, in particular significant laboratory abnormality indicative of hepatic condition (more than 3 times the upper limit)
4. *Allergy*: ascertained or presumptive hypersensitivity to the active principle and/or formulations' ingredients; history of anaphylaxis to drugs or allergic reactions in general, which the investigator considers may affect the outcome of the study
5. *Diseases*: significant history of renal, hepatic, gastrointestinal, cardiovascular, respiratory, skin, haematological, endocrine, urologic, metabolic, neurological or psychiatric diseases, as determined by the investigator, that may interfere with the aim of the study; history of carcinoma *in situ* and malignant disease; active bacterial or viral infection and fever >38° C within 48 h prior to study treatment administration
6. *Virology*: positive result of HIV, hepatitis B (HBV), hepatitis C (HCV) or *Treponema pallidum* (TP) assays
7. *Surgery*: any surgery within 60 calendar days of screening (excluding diagnostic surgery)
8. *Medications*: medications, including over the counter (OTC) medications, herbal remedies and traditional Chinese remedies for 2 weeks before the start of the study. Hormonal contraceptives for women will be allowed
9. *Investigative drug studies*: participation in the evaluation of any investigational product for 1 month before this study
10. *Blood donation*: blood donations for 90 calendar days before this study
11. *Drug, alcohol, caffeine, tobacco*: history of drug, alcohol [>1 drink/day for females and >2 drinks/day for males, defined according to the USDA Dietary Guidelines 2015-2020] caffeine (>5 cups coffee/tea/day) or tobacco abuse (≥10 cigarettes/day)
12. *Abuse drug test*: positive urine abuse drug test at screening or day -1
13. *Alcohol test*: positive alcohol breath test at day -1
14. *Diet*: abnormal diets (<1600 or >3500 kcal/day) or substantial changes in eating habits in the 4 weeks before this study; vegetarians; consumption of alcohol, grapefruit, products containing grapefruit, or beverages containing xanthines (coffee, tea, soda, coffee with milk, energy drinks) within 48 hours prior to the enrolment
15. Pregnancy (females only): positive or missing pregnancy test at screening or day -1, pregnant or lactating women
16. Vaccination within 4 weeks of study treatment
17. Other unspecified reasons that, in the opinion of the investigator, make the subject unsuitable for enrolment.

10.3.1 Not allowed treatments

No medication, including OTC, traditional Chinese medicine and herbal remedies, will be allowed for 2 weeks before the start of the study and during the whole study duration. Paracetamol will be allowed as therapeutic counter-measure for adverse events (AEs) according to the investigator's opinion. Hormonal contraceptives will be allowed.

The intake of any other medication will be reported as a protocol deviation.

11 STUDY SCHEDULE

The schedule of the study is summarised at page 11.

11.1 Study visits and procedures

Each study subject will undergo 6 visits.

The study protocol foresees a screening visit and a confinement lasting from Visit 2 to the Final Visit/early termination visit (ETV), i.e. from Day -1 to Day 8. Maximum study duration will be 22 days, screening visit included. A written informed consent will be obtained before any study assessment or procedure.

The first subject first visit (FSFV) is defined as the 1st visit performed at the clinical centre on the 1st screened subject. The last subject last visit (LSLV) is defined as the last visit performed at the clinical centre (or the telephonic follow-up, if applicable) on the last subject, i.e. the last visit foreseen by the study protocol, independently of the fact that the subject is a completer or a withdrawn subject.

The following phases, visits and procedures will be performed:

- **Screening phase**
  - Screening – visit 1: between day -14 and day -2
  - Visit 2: day -1

- **Interventional phase**
  - Visit 3: days 1-3
  - Visit 4: days 4-5
  - Visit 5: days 6-7

- **Final phase**
  - Visit 6: day 8 - Final visit/ETV. In case of early discontinuation, discontinued subjects will undergo an ETV

Activities to be performed are listed by visit in the following table:

Table 11.1.1 Schedule of study activities and procedures

	Day	Procedures/Assessments	Notes
Screening – Visit 1	*From day -14 to day -2*	➢ Explanation to the subject of study aims, procedures and possible risks ➢ Informed consent signature ➢ Screening number (e.g. [redacted], etc.) ➢ Demographic data and life style recording ➢ Medical/surgical history ➢ Previous/concomitant medications ➢ Full physical examination (body weight, height, vital signs, physical abnormalities) ➢ ECG recording ➢ Clinical laboratory analyses: haematology, blood chemistry, urinalysis, serum virology, bacteriology and pregnancy test (women) ➢ Urine multi-drug kit test ➢ AE monitoring ➢ Inclusion/exclusion criteria evaluation ➢ Eligibility evaluation	The subject screening numbers (e.g. [redacted], etc.) will be used to identify the subjects
Visit 2	*Day -1*	➢ Alcohol breath test ➢ Urine multi-drug kit test ➢ Urine pregnancy test (women) ➢ Inclusion/exclusion criteria evaluation ➢ Eligibility evaluation ➢ Enrolment ➢ Study subject number will be the same as the screening number that was initially assigned when the subject is successfully enrolled (e.g screening number .) ➢ Check of AEs and concomitant medications	Arrival at the clinic before the evening (before 18:30) Confinement until the morning of day 8 Standardised dinner Fasting for at least 10 h (overnight) before first dosing
Visit 3	*Day 1*	➢ Vital sign measurement at pre-dose ➢ IMP administration at 08:00 ± 1 h; intravenous infusion for 5 min ➢ Vital signs measurement at 1 h post-dose ➢ Blood sample collection for pharmacokinetic analysis at: pre-dose (0) and 5 (at the end of the infusion), 8, 12, 15, 20, 25, 30, 60 min, 2, 4, 6, 8, 10 and 12 h post-dose ➢ Urine sample collection for pharmacokinetic analysis at: 0-4; 4-8; 8-12 and 12-24 h post-dose ➢ Check of AEs and concomitant medications	Standardized lunch at 13:00 standardized dinner at 21:00
	Day 2	➢ Blood sample collection for pharmacokinetic analysis at 24 and 32 h post-dose ➢ Urine sample collection for pharmacokinetic analysis at 24-32 h post-dose ➢ Check of AEs and concomitant medications	Standardized breakfast around 9:00 Standardized lunch around 13:00 Standardized dinner around 21:00
	Day 3	➢ Vital sign measurement at 48 h post-dose ➢ Physical examination (body weight, physical abnormalities) at 48 h post-dose ➢ Clinical laboratory analyses: haematology, blood chemistry, urinalysis at 48 h post-dose ➢ Check of AEs and concomitant medications	Standardized breakfast around 9:00 Standardized lunch around 13:00 Standardized dinner around 21:00

	Day	Procedures/Assessments	Notes
Visit 4	*From day 4 to day 5*	➢ IMP administration at 08:00 ± 1 h; infusion for 5 min ➢ IMP administration at 20:00 ± 1 h; infusion for 5 min ➢ Blood sample collection for pharmacokinetic analysis at: pre-dose (0) before the morning infusion ➢ Check of AEs and concomitant medications	Standardized breakfast around 9:00 Standardized lunch around 13:00 Standardized dinner around 21:00
Visit 5	*Day 6*	➢ Vital sign measurement at pre-dose ➢ IMP administration at 08:00 ± 1 h; infusion for 5 min ➢ Vital signs measurement at 1 h post-dose ➢ Blood sample collection for pharmacokinetic analysis at: pre-dose (0) and 5 (at the end of the infusion), 8, 12, 15, 20, 25, 30, 60 min, 2, 4, 6, 8, 10 and 12 h post-dose ➢ Urine sample collection for pharmacokinetic analysis at: 0-4; 4-8; 8-12 and 12-24 h post-dose ➢ Check of AEs and concomitant medications	Standardized lunch at 13:00 (about 5 h post-dose) Standardized dinner at 21:00 (about 13 h post-dose)
	Day 7	➢ Blood sample collection for pharmacokinetic analysis at 24 and 32 h post-dose ➢ Urine sample collection for pharmacokinetic analysis at 24-32 h post-dose ➢ Check of AEs and concomitant medications	Standardized breakfast around 9:00 Standardized lunch around 13:00 Standardized dinner around 21:00
Final Visit/ETV	*Day 8 or early termination visit (ETV) in case of discontinuation*	➢ Vital sign measurement at 48 h post-dose (or at ETV) ➢ Physical examination (body weight, physical abnormalities) ➢ Clinical laboratory analyses: haematology, blood chemistry, urinalysis ➢ Check of AEs and concomitant medications In case of clinically significant results at the final visit, the subjects will be followed-up by the investigator until the normalisation of the concerned clinical parameter(s)	Discharge from the clinical centre in the morning of Day 8 after vital sign measurement, blood sampling for clinical laboratory assays and physical examination or in case of ETV. Upon leaving, the subjects will be instructed to contact immediately the investigator in case of occurrence of any untoward medical occurrence

11.2 Diet and lifestyle

The subjects will not take any food or drinks (except water) for at least 10 h (i.e. overnight) before the first drug administration (Day 1) and will remain fasted up to 5 h post-dose.
During the study, water will be allowed as desired. In order to maintain an adequate hydration, the subjects will be encouraged to drink at least 120 mL of mineral water every 2 h for 6 h post-dose on Days 1 and 6.

Coffee, tea or food containing xanthines (i.e. coffee, tea, soda, coffee with milk, energy drinks, etc.), alcohol and grapefruit will be forbidden starting 48 h before the enrolment until the end of the study. Smoking is not allowed for the whole study duration.

During confinement, routine ambulant daily activities will be strongly recommended.

The timing of each meal to be served during confinement is shown in Table11.1.1 above.
In the evening of day -1, upon confinement, the subjects will receive a light dinner before fasting overnight.
On days 1 and 6 (PK sampling collection days), standardised lunch and dinner will be served.
On days 2, 3, 4, 5 and 7, standardised breakfast, lunch and dinner will be served.

11.2.1 Restrictions

During the study, the subjects will be confined from the evening preceding the first drug administration (study day -1) until the morning of day 8 (after the final visit). They will attend the clinic in the evening of day -1 not later than 18:30.

During confinement, hazardous, strenuous or athletic activities will not be permitted.

12 DESCRIPTION OF SPECIFIC PROCEDURES

12.1 Physical examination

Full physical examinations will be performed at the screening visit, visit 3, day 3 and final visit/ ETV. Information about the physical examination will be recorded by the investigator. Any abnormalities will be recorded.

Significant findings/illnesses, reported after the start of the study and that meet the definition of an AE (see § 16), will be recorded in the subject source documents.

Date of the physical examination, overall investigator's interpretation (as normal or abnormal and, if abnormal, clinically significant or not clinically significant) and clinically significant abnormalities (if any) will be reported in the individual CRFs.

12.1.1 Body weight

Body weight will be recorded during each physical examination.
Subjects will be weighed (kg) lightly clothed without shoes. Height will be measured at screening only and BMI will be recorded. BMI will be calculated as weight [kg]/(height [m] x height [m]).

12.1.2 Vital signs

Subjects blood pressure (BP) and heart rate (HR) will be measured by the investigator or his/her deputy after 5 min at rest (in sitting position) at:

- Screening
- Visit 3, Day 1: at pre-dose and 1 h post-dose
- Visit 3, Day 3: at 48 h post-dose
- Visit 5, Day 6: at pre-dose and 1 h post-dose
- Final visit (at 48 h post-dose)/ETV

12.1.3 ECGs

One 12-lead ECG will be performed (in sitting/supine position) at screening only.

12.2 Clinical laboratory assays

Samples of blood and urine will be collected. The following laboratory analyses will be performed at the screening visit:

Haematology
Leukocytes and leukocyte differential count (percentage values and absolute values), erythrocytes, haemoglobin (conv. units), haemoglobin (IS units), haematocrit, MCV, MCH, MCHC, thrombocytes.

Blood chemistry
Electrolytes: sodium, potassium, calcium, chloride, inorganic phosphorus
Enzymes: alkaline phosphatase, γ-GT, AST, ALT
Substrates/metabolites: total bilirubin, creatinine, glucose, BUN or urea, uric acid, total cholesterol, triglycerides
Proteins: total proteins

Serum pregnancy test (women).

Urine analysis
pH, specific gravity, appearance, colour, nitrites, proteins, glucose, urobilinogen, bilirubin, ketones, blood, leukocytes, erythrocytes, epithelial cells, crystals, cylinders.

Serum virology and bacteriology
Hepatitis B (HBs antigen), **Hepatitis C** (HCV antibodies), **HIV 1/2** (HIV Ag/Ab combo), *Treponema pallidum*.

The same analyses, with the exception of virology, bacteriology and serum pregnancy test, will be performed also at Visit 3, Day 3 (48 h post-dose after the single dose) and at the final visit/ETV.

A urine drug test will be performed at the clinical centre at screening, using a urine multi-drug kit. The following drugs will be assessed: cocaine, amphetamine, methamphetamine, cannabinoids (delta-9-tetrahydrocannabinol - THC), opiates. The same test will be repeated upon confinement at Visit 2, day -1.

A serum pregnancy test will be performed by the laboratory at screening. A urine pregnancy test will be performed at Visit 2, day -1 at the clinical centre, upon confinement.

Date/time of samples collection, overall investigator's interpretation (as normal or abnormal and, if abnormal, clinically significant or not clinically significant) and clinically significant findings (if any) will be reported in the individual CRFs. All clinically significant abnormalities after the screening visit will be recorded as AEs.

12.3 Sampling for pharmacokinetic analysis

12.3.1 Venous blood sampling

Venous blood samples (up to 10 mL) will be collected from a forearm vein at the following times, both on Day 1, 2, after the single dose, and on Day 6, 7, after the last multiple dose:

- at pre-dose (0) and 5 (at the end of the infusion), 8, 12, 15, 20, 25, 30, 60 min, 2, 4, 6, 8, 10, 12, 24 and 32 h post-dose

The start of infusion (not the end) will be considered as time 0.

Actual sampling times for each subject will be recorded in the individual case report forms (CRFs). The actual sampling times should not exceed the recommended tolerance ranges presented in the following table. Any deviation outside the recommended ranges will be verified

through Data Clarification Forms and, if confirmed, will be reported as protocol deviation, although it will not automatically lead to the exclusion of the concerned subjects from the PK analysis set.

Table 12.3.1.1 Tolerance ranges for the scheduled sampling times

Sampling time	Tolerance range
Pre-dose (0)	Within 30 minutes before IMP administration
0.0833 h (5 min), 0.1333 (8 min) 0.200 h (12 min), 0.250 h (15 min)	20 seconds
0.3333 h (20 min), 0.4167 (25 min), 0.5 h (30 min)	± 1 min
1 h	± 3 min
2, 4 h	± 5 min
6, 8, 10, 12, 24, 32 h	± 10 min

Blood samples for PK analysis will be collected using an indwelling catheter with switch valve. The cannula will be rinsed, after each sampling, with about 1 mL of sterile saline solution. The first mL of blood will be discarded at each collection time.

The remaining blood sample will be collected into blood collection tube.

Storage on ice will not last longer than 60 min until centrifugation at 4° C for 10 min at 2500xg to obtain plasma. Plasma will be collected into separate tubes, where an appropriate volume of DTT solution (50 µL DTT solution/mL plasma, i.e. 5% of the plasma volume according to the ratio 75 µL of DTT solution for 1.5 mL of plasma) will be added. The samples will be mixed shortly (example Vortex, 20 sec or relevant method to mix evenly inverting the tube up and down gently ~ about 20 times), divided into two aliquots, P1 and P2 and transferred to ≤-70° C as soon as possible. The DTT solution (5 mg DTT/mL of water) will be freshly prepared in the evening before each sampling day and kept refrigerated until use. The preparation of the solution will be recorded in appropriate forms.

If any clinical assessment, such as vital signs measurement, is foreseen at the same time-point as blood sampling for PK analysis, blood collection will be performed at the scheduled time. However, vital signs can be influenced by the blood sampling. Therefore, these assessments can be performed within 30 min before the pre-dose PK time point (0 h) and within 10 min before the other scheduled PK time-points. Any deviations outside the recommended time will be verified through Data Clarification Forms. However, since vital signs measurements will be performed for safety reasons only, deviations from the planned time schedule will be considered not relevant.

12.3.2 Urine collection

Urine for PK will be collected in the following time intervals both on Day 1, 2, after the single dose, and on Day 6, 7 after the last multiple dose:

- 0-4; 4-8; 8-12, 12-24 and 24-32 h post-dose

Bladder must be emptied before the end of each collection period. During each interval, urine will be collected into containers, kept refrigerated at approximately 4° C and containing DTT solution. At the end of each collection interval, urine volume will be measured and after

thorough mixing, two aliquots of 1 mL each (U1 and U2) will be prepared in polypropylene tubes. Urine volume collected in each collection interval will be accurately recorded in appropriate forms and in the CRFs. The two aliquots will be stored at ≤-70° C. The DTT solution (5 mg DTT/mL of water) will be freshly prepared in the evening before each sampling day and kept refrigerated until use. The preparation of the solution will be recorded in appropriate forms.

12.3.3 Analytics

The concentration of total NAC in plasma and urine will be determined at a certified bioanalytical laboratory to be designated in China, using a fully validated LC-MS/MS method, with a lower quantification limit (LQL) of 10 ng/mL.

Analyses will be performed according to the general Principles of "OECD Good Laboratory Practices for testing of chemicals" C(81) 30 (final) and GCP/ CFDA GCP (see also § 9.2).

The method validation report and the analytical report will be attached to the final report.

12.3.4 Labelling, storage and transport of samples

12.3.4.1 Samples labelling

Each sample tube will be clearly and unequivocally identified with a label resistant to the storage temperature and reporting:

Study code	Sponsor code Z7244J01
Subject number	Subject or Process or Unique Subject Identification number, e.g. CCI, etc.
Tube identification	CCI
Study day	1 or 6
Scheduled sampling time	as min and h; see § 12.3.1

12.3.4.2 Samples storage and transport

During the study the samples will be stored at ≤-70° C. At the end of each collection day, aliquots 1 and 2 will be stored in separate freezers.

All aliquots 1, packed in sufficient solid CO_2, will be shipped by an authorised courier from the clinical site, China, to Eurofins Central Laboratory, Shanghai, China. Aliquots 1 will remain stored at Eurofins Central Laboratory or a subcontracted bioanalytical laboratory to be designated until finalisation of the bioanalytical report. Afterwards, the samples will be destroyed and a certificate of destruction will be provided to the sponsor.

The counter-samples (aliquot 2) will remain stored at the clinical site, China. These samples could either be:

- sent to the laboratory for reanalysis should this become necessary for analytical reasons or if any problems occur during the delivery of aliquots 1, or
- destroyed at an authorised site, or

- transferred to the sponsor upon written request.

No analyses different from those stated in this protocol and agreed by the subjects when signing the informed consent form will be performed unless a new informed consent and a new approval from the Ethical Committee is obtained. The subjects may ask to destroy their own samples at any time.

13 ASSIGNMENT OF STUDY TREATMENT

13.1 Randomisation

No randomisation will take place in the present study. All the subjects will receive the same treatment.

13.2 Blinding

This is an open study. No masking procedure will be applied.

14 EVALUATION PARAMETERS

14.1 Study variables

14.1.1 Primary variables

After single dose:

- C_{max}, t_{max}, k_{el}, $t_{1/2}$, $AUC_{(0-t)}$, $AUC_{(0-\infty)}$, $AUC_{(0-12)}$, V_d, CL_t, $Ae_{(0-t)}$, $Fe_{(0-t)}$ and CL_r

After multiple doses:

- C_{ss_max}, t_{ss_max}, C_{ss_min}, $AUCss_{(0-t)}$, $AUCss_{(0-12)}$, C_{ss_av}, R, DF% and $Aess_{(0-t)}$.

14.1.2 Secondary variables

- TEAEs, vital signs (BP, HR), body weight, physical examinations, laboratory parameters.

14.2 Pharmacokinetic assessments

14.2.1 Pharmacokinetic parameters

The following PK parameters will be measured and/or calculated for plasma NAC, using the validated software Phoenix® WinNonlin® version 6.3 or higher (Certara USA, Inc., Princeton, NJ) (actual version will be stated in the final report), after single dose of IMP:

C_{max}:	Maximum NAC plasma concentration
t_{max}:	Time to achieve C_{max}
k_{el}:	Terminal elimination rate constant, calculated, if feasible, by log-linear regression using at least 3 points
$t_{1/2}$:	Half-life, calculated, if feasible, as $ln2/k_{el}$
$AUC_{(0-t)}$:	Area under the concentration-time curve from single dose to the last observed concentration time t, calculated with the linear up/log down trapezoidal method
$AUC_{(0-\infty)}$:	Area under the concentration-time curve extrapolated to infinity, calculated, if feasible, as $AUC_{(0-t)} + C_t/k_{el}$, where C_t is the last measurable drug concentration
$AUC_{(0-12)}$:	Area under the concentration-time curve at steady-state in the tau interval (from single dose to 12 h post-dose), calculated with the linear up/log down trapezoidal method

V_d: Volume of distribution associated with the terminal slope, calculated, if feasible, as Dose/($AUC_{(0-\infty)}$*k_{el})

CL_t: Total body clearance, calculated, if feasible, as Dose/ $AUC_{(0-\infty)}$

$Ae_{(0-t)}$: Total amount of NAC excreted in urine from single dose up to 32 h

$Fe_{(0-t)}$: Total fraction of NAC dose excreted in urine from single dose up to 32 h

CL_r: Renal clearance, calculated, if feasible, as $Ae_{(0-t)}$/ $AUC_{(0-\infty)}$

The following PK parameters will be measured and/or calculated for plasma NAC, using the same software, after multiple doses of IMP:

C_{ss_max}: Maximum NAC plasma concentration at steady-state

t_{ss_max}: Time to achieve C_{ss_max}

C_{ss_min}: Trough NAC plasma concentration at steady-state, measured as concentration at t=12 h

$AUCss_{(0-t)}$: Area under the concentration-time curve at steady-state from the last multiple dose to the last observed concentration time t, calculated with the linear up/log down trapezoidal method

$AUCss_{(0-12)}$: Area under the concentration-time curve at steady-state in the tau interval (from the last multiple dose to 12 h post-dose), calculated with the linear up/log down trapezoidal method

C_{ss_av}: Average NAC plasma concentration at steady-state, calculated as $AUCss_{(0-12)}$ /tau

R: Accumulation ratio, calculated as $AUCss_{(0-12)}$/ $AUC_{(0-12)}$

$Aess_{(0-t)}$: Total amount of NAC excreted in urine from the last multiple dose to 32 h at steady-state

DF%: Degree of fluctuation over one dosing interval at steady-state, calculated as (C_{ss_max} - C_{ss_min})/ C_{ss_av}*100

The sampling schedule is considered adequate if the ratio $AUC_{(0-t)}$/ $AUC_{(0-\infty)}$ equals or exceeds a factor of 0.8 (i.e. if $\%AUC_{extra}$ is <20%) for more than 80% of the individual PK profiles. This assures that the primary variable $AUC_{(0-t)}$ covers a sufficient percentage of the theoretical total extent of exposure.

The quality of log-linear regression (and, consequently, the reliability of the extrapolated PK parameters) should be demonstrated by a determination coefficient $R^2 \geq 0.8$. Individual extrapolated parameters, when considered unreliable, will be reported as NC (not calculated).

14.3 Safety assessments

Safety and general tolerability of the IMP will be based on TEAEs, physical examinations including body weight, vital signs and routine haematology, blood chemistry and urinalysis laboratory tests.

15 STATISTICAL METHODS

The data documented in this trial and the measured clinical parameters will be presented using classic descriptive statistics (i.e. total number of subjects treated [N], number of observations [n], mean standard deviation [SD], minimum [Min], median, maximum [Max]) for quantitative variables and frequencies (i.e. count and percentages) for qualitative variables if not stated otherwise.

Not available data will be evaluated as "missing values". The statistical analysis of demographic and safety data will be performed using the analysis software SAS® .

The statistical analysis of PK parameters will be performed using the validated software Phoenix® WinNonlin® version 6.3 or higher (Certara USA, Inc., Princeton, NJ) (actual version will be stated in the final report).

15.1 Analysis Sets

15.1.1 Definitions

A subject will be defined as screened after the signature of the informed consent, regardless of the completion of all the screening procedures.

A subject will be defined as eligible if he/she meets all the inclusion/exclusion criteria. Otherwise he/she will be defined as a screen failure.

A subject will be defined as enrolled in the study if he/she is included into the interventional phase of the study.

The following analysis sets are defined:

- Enrolled set: all enrolled subjects. This analysis set will be used for demographic, baseline and background characteristics
- Safety set: all subjects who receive at least one dose of IMP. This analysis set will be used for the safety analyses
- PK set: all enrolled subjects who fulfil the study protocol requirements in terms of IMP intake and have evaluable PK data readouts for the planned analyses, with no major deviations that may affect the PK results. This analysis set will be used for the statistical analysis of the PK parameters.

Each subject will be coded as valid or not valid for the safety and the PK set. Subjects will be evaluated according to the treatment they actually receive.

15.1.2 Reasons for exclusion from the PK set before bioanalysis

Reasons for the exclusion of subjects from the PK set are the following:

- intake of concomitant medications which could render the plasma concentration-time profile unreliable
- AEs which could render the plasma concentration-time profile unreliable
- administration errors which could render the plasma concentration-time profile unreliable
- other events which could render the plasma concentration-time profile unreliable

If one of these events occurs, it will be noted in the CRF during the study.

The samples from the subjects excluded from the PK set should still be assayed and the results listed. Subjects should not be excluded from the PK set if the AUC_{0-t} covers less than 80% of the $AUC_{0-\infty}$.

15.2 Sample size and power considerations

The sample size was not calculated through any statistical calculation. A sample size of 24 subjects was estimated as sufficient for the descriptive purposes of the present study.
Drop-out subjects will not be replaced.

15.3 Demographic, baseline and background characteristics

Critical demographic characteristics will be examined according to qualitative or quantitative data. Qualitative data will be summarised in contingency tables. Quantitative data will be summarised using classic descriptive statistics.

15.4 Analysis of pharmacokinetic parameters

15.4.1 Descriptive pharmacokinetics

A descriptive PK will be presented. The results will be displayed and summarised in tables and figures. Individual and mean curves (+SD at sampling times), indicating inter-subject variability, will be plotted. Data below the lower quantification limit (BLQL) will be considered as 0 in the calculations and presented as BLQL in listings and tables. As a consequence of BLQL (i.e. 0) values, calculated geometric means (if requested) could be null. For this reason, in the presence of any null value, the geometric mean will be reported as not calculated (NC).

15.5 Safety and tolerability evaluation

- **AEs**

AEs will be coded by System Organ Class (SOC) and Preferred Term (PT), using the Medical Dictionary for Regulatory Activities (MedDRA).
AEs will be classified as pre-treatment AEs (PTAEs) and TEAEs, according to the period of occurrence, as follows:

- PTAEs: all AEs occurring before the first dose of IMP and not worsening after the first dose of IMP
- TEAEs: all AEs occurring or worsening after the first dose of IMP

Individual PTAEs and TEAEs will be listed in subject data listings. No summary table will be provided for PTAEs. TEAEs will be summarised by treatment and overall. The number and percentage of subjects with any TEAE and the number of TEAEs will be tabulated by SOC and PT, seriousness, relationship to treatment and severity.

- **Physical examination**

Date of the physical examination, overall investigator's interpretation (as normal or abnormal and, if abnormal, clinically significant or not clinically significant) and clinically significant abnormalities (if any) will be listed.

- **Laboratory data**

Date/time of samples collection, overall investigator's interpretation (as normal or abnormal and, if abnormal, clinically significant or not clinically significant) and clinically significant findings (if any) will be listed. All laboratory results will be listed and a table of all the abnormal values will be presented. The overall investigator's interpretation will be summarised using tables of frequency.
The toxicity grading of laboratory tests is determined based on the NCI CTCAE V4.03.

- **Vital signs**

Vital signs values will be listed and summarised by descriptive statistics.

- **Body weight**

Body weight values will be listed and summarised by descriptive statistics.

16 DEFINITION AND HANDLING OF AEs AND SAEs

16.1 Applicable SOPs

AEs definition, classification and management will follow the CRO's SOPs, based upon applicable local and international regulations. The full SOPs or an operative summary will be made available to the clinical centre.

A brief summary of AE definition, classification and management is reported below.

16.2 Definition of Adverse Event (AE)

An Adverse Event is "*any untoward medical occurrence in a patient or clinical trial subject administered a medicinal product and which does not necessarily have a causal relationship with this treatment*".

AEs include:

- worsening (change in nature, severity or frequency) of conditions present at the onset of the trial
- patient/subject deterioration due to the primary illness
- intercurrent illnesses
- drug interactions
- events related or possibly related to concomitant medications
- abnormal laboratory values, as well as significant shifts from baseline within the range of normal, which the investigator considers to be clinically significant.

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

16.3 Definition of Adverse Drug Reaction (ADR)

An Adverse Reaction is "*any untoward and unintended response to an investigational medicinal product related to any dose administered*".
All AEs judged by either the reporting investigator or the sponsor as having a reasonable causal relationship to a medicinal product qualify as adverse reactions. The expression reasonable causal relationship means to convey in general that there is evidence or matter to suggest a causal relationship.

- **Unexpected Adverse Drug Reaction**

An unexpected ADR is: "*An adverse reaction, the nature, or severity of which is not consistent with the applicable product information (Reference Safety Information [RSI])*.

- **Reference Safety Information (RSI)**: in order to assess whether an adverse reaction is expected, the IB will be used.

16.4 Definition of Serious Adverse Events or Serious Adverse Drug Reaction

A Serious Adverse Event (SAE) is: "*any untoward medical occurrence or effect that at any dose*:

- results in death
- is life-threatening (i.e. the subject was at risk of death at the time of the event/reaction; it does not refer to an event/reaction which hypothetically might have caused death if it were more severe)
- requires inpatient hospitalization or prolongation of existing hospitalization
- results in persistent or significant disability/incapacity (where disability is defined as a permanent or substantial disruption of ability to carry out normal life functions, either reported or defined as per clinical judgement)
- is a congenital anomaly/birth defect
- is an important medical event that may not result in death, be life-threatening, or require hospitalization but, according to appropriate medical judgment, it may jeopardize the subject and may require medical or surgical intervention to prevent any of the outcomes listed in the definition above. Examples of such events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, and blood dyscrasias or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse.

Any suspected transmission via a medicinal product of an infectious agent is also considered a serious adverse event.

A **Suspected Unexpected Serious Adverse Reaction** (SUSAR) is an ADR that is both unexpected (not consistent with the applicable product information, e.g. IB) and also meets the definition of a SAE.

A non-serious adverse event is any adverse event that does not meet the criteria listed above for a serious adverse event.

16.5 Definition of Severity of Adverse Events

The term "severe" is used to describe the intensity (severity) of a specific event:

- Mild: causing no limitation of usual activities; the subject may experience slight discomfort.
- Moderate: causing some limitation of usual activities; the subject may experience annoying discomfort.
- Severe: causing inability to carry out usual activities; the subject may experience intolerable discomfort or pain.

16.6 Definition of Adverse Event causality

Causality shall be determined according to the definition of ADR given in 16.3.

All AE judged by either the investigator or the sponsor as having a reasonable suspected causal relationship to an investigational medicinal product qualify as adverse reactions. The causality assessment given by the investigator should not be downgraded by the sponsor.

The following binary decision for causality will be used:

- Reasonable possibility that the IMP caused the event
- No reasonable possibility that the IMP caused the event

Features supportive of an association include:

- temporal plausibility
- pharmacological properties of the drug or of the substance class
- course of the adverse event after dechallenge and, if applicable, after rechallenge
- specific tests indicating involvement of the drug in the occurrence/worsening of the adverse event
- alternative explanations

16.7 Adverse Events recording

Each AE occurring to a subject, either spontaneously revealed by the subject or observed by the investigator, whether believed by the investigator to be related or unrelated to the IMP, must be recorded on the AE information page of the eCRF (for SAEs information must be recorded also on the AE information page of the eCRF).
The Investigator will also perform an evaluation with respect to seriousness and causality (relationship of any AE to IMP) of the AEs. and record it on the appropriate section of the eCRF and records it on the appropriate section of the eCRF.

16.8 AEs monitoring window

- Start of monitoring: from immediately after the signature of the informed consent
- End of monitoring: final study visit/ETV (observation window)

An AE occurring after the final visit/ETV and coming to knowledge of the investigator (e.g. during a follow-up assessment or by spontaneous reporting by study subjects) must be recorded only if it is an ADR, according to the investigator's judgment.

16.9 Adverse Events reporting

The official language for reporting is English. The investigator and clinical staff of the present study are familiar with English language.

The investigator must report to the CRO all AEs which occur during the study, regardless of their relationship to the IMP. Protocol specific AEs or laboratory abnormalities critical to safety evaluations are to be identified in the protocol and reported to the sponsor according to reporting requirements and within the time periods specified.

All AEs are recorded by the investigator on the AE information page of the eCRF.

In addition, SAE will have to be reported according to the following detailed procedure.

16.9.1 SAEs reporting

With the exception of those SAEs that are identified as not requiring immediate reporting in the protocol, the investigator must report the SAEs to the CRO immediately and no later than 24 hours from when he/she becomes aware of the SAE, by faxing the "Serious Adverse Event Form" (back up plan) or e-mailing as scanned attachment (backup plan) or by Electronic Data Capture to the Drug Safety Unit (DSU) personnel (preferred method), as stated in the "List of CRO/ personnel" in § 16.13 of this protocol.

The community standards of confidentiality must always be maintained and any relevant national legislation on data protection must be followed.

Another copy of the "Serious Adverse Event Form" will be retained by the Investigator for the Investigator's file.

If the investigator becomes aware of any SAE occurred to a subject within the follow-up window established in the protocol, he/she will report the SAE as above. The SAE will be also reported in the eCRF.

If outside the follow-up window established in the protocol the investigator becomes aware of a SAE, if the investigator judges that the SAE is related to the study drug, it should be reported to the Sponsor. The Investigator might use the "Serious Adverse Event Form" via email or fax, but the SAE must not to be reported in the eCRF, as it is not an event occurred within the study period.

The investigator must report all SAEs that occur to the subjects to National Medical Products Administration/Local Ethics Committee/Provincial Food and Drug Administration/National Health and Family Planning Commission immediately no later than 24hours from when he/she becomes aware of SAE. Any SAEs that happen to the subjects outside the follow-up window should be reported by investigator to National Medical Products Administration/Local Ethics Committee/Provincial Food and Drug Administration/National Health and Family Planning Commission immediately.

16.10 Follow-up for Adverse Events

A follow-up "Serious Adverse Event Form" will be filled in if important follow-up information (i.e. diagnosis, outcome, causality assessment, results of specific investigations) is made available after submission of the initial AE Form for immediate reporting. Follow-up "Serious Adverse Event Form" will be reported to the Sponsor as above-described, under Section 16.9.1.

In any case of an AE that, in the opinion of the investigator, requires the subject's discontinuation, follow-up information relating to the subject's subsequent course must be collected until the event has subsided or the condition stabilised or when the subject is lost to follow up or death.

When follow-up data on non-serious AE are collected, information should be reported under "Comments" in the Final report of the CRF.

16.11 SUSARs management

The clock for initial expedited reporting starts as soon as the information containing the minimum reporting criteria has been received by the sponsor (day 0).
For fatal and life-threatening SUSARs the EC and Competent Authority (CA) should be informed as soon as possible and in any case within 7 days.
If the initial report is incomplete, e.g. not all the information/assessments were available, a complete report should be sent within an additional 8 days. Complete follow-up report should be sent within 15 calendar days.
SUSARs which are not fatal and not life-threatening are to be reported within 15 days.
The minimum information to be reported includes:

- Sponsor study number
- One identifiable coded subject
- One identifiable reporter
- One SUSAR
- One suspect IMP (including active substance name, code)
- A causality assessment (a reasonable possibility of a causal relationship with the study drug can be excluded only if there is information supporting this decision, otherwise it cannot be excluded).

16.12 Other events qualified for expedited reporting

Other safety issues also qualify for expedited reporting when they might materially alter the current benefit-risk assessment of a medicinal product or would be sufficient to consider changes in the medicinal product administration or in the overall conduct of the trial, for instance:

- single case reports of an expected serious adverse reaction with an unexpected outcome (e.g. a fatal outcome)
- an increase in the rate of occurrence of an expected serious adverse reaction, which is judged to be clinically important.
- post-study SUSARs that occur after the subject has completed a clinical trial and are reported to the investigator by the subject.
- new events relating to the conduct of the trial or the development of the medicinal product likely to affect the safety of the subjects, such as :
  - a SAE which could be associated with the trial procedures and which could modify the conduct of the trial
  - a significant hazard to the subject population such as lack of efficacy of a medicinal product used for the treatment of a life-threatening disease
  - a major safety finding from a newly completed animal study (such as carcinogenicity) or from other clinical trials.

16.13 SAEs: contacts

The investigator will report any SAE to the CRO. The CRO's details for SAEs are the following:
Email: [redacted]

The sponsor's details for SAEs are the following:
Email: [redacted]

16.14 Pregnancy

Subjects must be instructed that known or suspected pregnancy occurring during the study should be confirmed and reported to the Investigator, who must then withdraw the subject from the study without delay. The Investigator should also be notified of pregnancy occurring during the study but confirmed after its completion. In the event that a subject is subsequently found to be pregnant after inclusion in the study, then any pregnancy will be followed to the end of pregnancy or pregnancy termination and the status of mother and child will be reported to the Sponsor after delivery through the Pregnancy Report Form provided by the Sponsor. The Investigator will send pregnancy reports to DSU within the timeframes of SAEs, with follow-up information to be actively sought for the outcome of pregnancy.

- Part I of the Form is completed at the time of awareness of foetal exposure during pregnancy.
- Part II of the Form is filled in when information on pregnancy outcome becomes available.

If pregnancy results in abnormal outcome (spontaneous miscarriage, stillbirth and congenital anomalies) that the investigator considers to be due to the IMP, this will be treated as an expedited ADR report.

17 DATA MANAGEMENT PROCEDURES

17.1 Data collection – CRFs

The investigator must ensure that the clinical data required by the study protocol are carefully reported in the eCRFs. He/she must also check that the data reported in the eCRFs correspond to those in the subject's source documents.
ECG and laboratory results must be printed and signed by the Investigator and kept as source data on site after entering outcome into the eCRF.
All other data has to be documented in the subject file as source data first and then entered into the eCRF.
Data must be entered into eCRFs in English by the designated site personnel as soon as possible after a subject visit, and monitors will have access to data recorded. These data will be reviewed versus source documents by trial monitors for completeness and acceptability during monitoring visits.
If data correction is required for an eCRF after initial entry, the site personnel can make necessary corrections on their own or as a response to an automated query by the eCRF system. Monitor and Clinical Data Manager review the eCRF for accuracy and can also generate queries to the investigational staff for resolution.
Any correction to the eCRFs' entries must be carried out by the Investigator or a designated member of staff. Corrections are recorded in an audit trail that records the old information, the new information, and identification of the person making the changes, date of correction made and reason for change. In the interests of completeness of data acquisition, the questions which are repeated in each section of the eCRFs should be answered in full, even if there are no changes from a previous examination. A reasonable explanation must be given by the Investigator for all missing data. The Investigator or his/her designees named in the clinical staff list will review the eCRF for accuracy and completeness. The Investigator must electronically sign and date the eCRF pages as indicated.

17.2 Database management

The CRO will update and verify the database and create the final SAS data sets. The final data file will be transferred to the sponsor in the agreed format with all the other study documentation.

17.2.1 Coding dictionaries

Medical/surgical history and underlying diseases, clinically significant physical examination abnormalities and AEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA™).
Previous and concomitant medications will be coded using the WHO Drug Dictionary Enhanced (WHODDE). The version of the coding dictionaries will be stated in the study report.

18 STUDY MONITORING, QUALITY CONTROL AND QUALITY ASSURANCE

18.1 Monitoring

The monitoring visits will be conducted by appropriate staff of PAREXEL China Co. Ltd according to PAREXEL SOPs.

Monitoring activities, including monitoring purpose, selection and qualifications of monitors, extent and nature of monitoring, monitoring procedures, monitoring reports will comply with ICH-GCP chapter 5.18, CFDA GCP requirements and, when appropriate, further national regulatory guidelines.

Adequate time and availability for monitoring activities should be ensured by the investigator and key study personnel.

Data verification is required and will be done by direct comparison with source documents, always giving due consideration to data protection and medical confidentiality. In this respect the investigator will assure support to the monitor at all times.

The investigator agrees, by written consent to this protocol, to fully co-operate with compliance checks by allowing authorised individuals to have access to all the study documentation. In addition to the monitoring activities performed by the study monitor, the sponsor could perform some quality control activities to verify the compliance with the study procedures, the ICH-GCP guidelines and CFDA GCP.

18.2 Quality Control and Quality Assurance

The CRO has implemented and maintains a Quality System that includes quality controls and audits at different study steps with written SOPs to ensure that the study is conducted in compliance with the protocol and all effective amendments, ICH-GCP, and the applicable regulatory requirement(s) and that data have been reliably and correctly generated, recorded, processed and reported, in agreement with the ALCOAC principles (Attributable-Legible-Contemporaneous-Original-Accurate-Complete).

The clinical site is responsible for implementing and maintaining quality assurance and a quality control system to ensure that the study is conducted, and data are generated, documented (recorded), and reported in compliance with the protocol, ICH-GCP, CFDA GCP and any applicable regulatory requirement(s).
This protocol has been audited by the Sponsor QA.
The CRO and the sponsor will be responsible for their respective activities.

The sponsor may transfer any or all of the sponsor's trial-related duties and functions to a CRO, but the ultimate responsibility for the quality and integrity of the trial data always resides with the sponsor.

18.3 Applicable SOPs

The sponsor, the clinical centre and the CRO will follow their respective SOPs in the conduct of the respective activities, unless otherwise stated in written agreements. CRO/Sponsor's SOPs will be used for AE and SAE definition and management. SOPs will be made available for Sponsor’s review, if required.

18.4 Data access

The investigator and the CRO will ensure that all source data, medical records, CRFs and all other documentation that is relevant to this study will be made accessible for monitoring activities, audits, IEC review, and regulatory inspections.

18.5 Audits and inspections

The sponsor, independent bodies acting on behalf of the sponsor and the CRO have the right to perform audits according to ICH-GCP, CFDA GCP and CFDA responsibilities.

The study may also be inspected by regulatory authorities.

The investigator and the CRO agree, by written consent to this protocol, to fully co-operate and support audits and inspections compliance checks by allowing authorised individuals to have access to all the study documentation.

19 ETHICAL CONSIDERATIONS

19.1 Ethics and Good Clinical Practice (GCP)

The study will be performed in accordance with the relevant guidelines of the Declaration of Helsinki.
The approval of the study protocol by the local IEC and by the Chinese Health Authorities will be obtained before the start of the study.

The present clinical study will be carried out according to the current revision of Good Clinical Practice (GCP), ICH topic E6 (R2), CFDA GCP and any applicable local law requirements.

19.2 Informed consent

Before being enrolled into the clinical study, the subjects must have expressed their consent to participate, after the investigator has explained to them, clearly and in details, the scope, the procedures and the possible consequences of the clinical study. Information will be given in both oral and written form. The information sheet and informed consent form will be prepared in the local language by the CRO and must be approved by the EC and regulatory authorities. It will include all the elements required by law according to the ICH-GCP and CFDA GCP recommendations.
In addition to the standard requirements that physicians are currently obliged to observe when providing information, the following points must also be covered:

- a description of the aims of the study and how it will be organised
- the type of treatment (information on the IMP and treatment procedures, as applicable)
- any potential negative effects attributable to the study product or treatment
- the freedom to ask for further information at any time
- the subjects' right to withdraw from the clinical study at any time without giving reasons and without jeopardising their further course of medical treatment
- the existence of a subject insurance cover and obligations following from this cover

Adequate time and opportunity to satisfy questions will be given to the subjects and the time will be recorded.

The investigator will be supplied with an adequate number of blank informed consent forms to be used. The forms will be signed and dated by both the investigator and the subject.
A copy of the signed form will be given to the subject.

To ensure medical confidentiality and data protection, the signed informed consent forms will be stored in the investigator's study file according to the regulatory requirements (see § 20.3).
The investigator will allow inspection of the forms by authorised representatives of the sponsor, EC members and regulatory authorities. He/she will confirm, by signing and dating the forms, that informed consent has been obtained.

19.3 Insurance policy

An insurance cover has been issued in favour of the subjects participating in this clinical study. The insurance is in compliance with the local regulation and with the requirements of the Health Authorities.

19.4 Withdrawal of subjects

It will be documented whether or not each subject completed the clinical study. If, for a subject, study treatment or observations are discontinued, the primary reason for discontinuation will be recorded.

19.4.1 Primary reason for discontinuation

- **Adverse event**: Any (significant) adverse event that in the opinion of the investigator or concerned subject is not compatible with study continuation. For the definition of AE, please refer to § 16.2.
- **death**: the absence of life or state of being dead
- **lost to follow-up**: the loss or lack of continuation of a subject to follow-up
- **non-compliance with study drug**: an indication that a subject has not agreed with or followed the instructions related to the study medication
- **physician decision**: a position, opinion or judgment reached after consideration by a physician with reference to the subject
- **pregnancy**: pregnancy is the state or condition of having a developing embryo or fetus in the body (uterus), after union of an ovum and spermatozoon, during the period from conception to birth
- **protocol deviation**: an event or decision that stands in contrast to the guidelines set out by the protocol
- **study terminated by sponsor**: an indication that a clinical study was stopped by its sponsor
- **technical problems**: a problem with some technical aspect of a clinical study, usually related to an instrument
- **withdrawal by subject**: study discontinuation requested by a subject for whatever reason
- **other**: different than the ones previously specified

For any subject discontinuing the study, the investigator will:

- ask the subject to undergo, as far as possible, a final medical visit (ETV) to examine the subject's health conditions and perform the required blood sampling for the laboratory assays. This examination will verify that all values tested at screening have remained within a clinically acceptable range (i.e. not clinically significant changes compared to screening)
- arrange for alternative medical care of the withdrawn subject, if necessary
- record the subject decision about the use of collected biological samples

- report in the CRF date and time of the last dose administration, and date and primary reason of study discontinuation
- record in the CRF any follow-up, if the subject is withdrawn for an AE

Discontinued subjects will not be replaced.

19.5 Study termination

The study will be considered terminated at the date of the last visit of the last subject or upon completion of any follow-up procedure described in protocol. The investigator and the sponsor have the right to discontinue the study at any time for reasonable medical and/or administrative reasons. As far as possible, this should occur after mutual consultation. Reasons for discontinuation have to be documented appropriately.

20 ADMINISTRATIVE PROCEDURES

20.1 Material supplied to the clinical centre

Beside IMP, the following study material will be supplied to the clinical centre:

- final version of the study protocol
- CRF for each subject plus some spare copies
- copy of the investigator's brochure (IB) relative to the IMP
- informed consent forms

Moreover, before the start of the study, the investigator will be provided with the following documents: CFDA GCP, ICH guidelines, confidentiality agreement (if applicable), protocol amendments (if any), declaration of Helsinki, insurance statement, SAE forms, financial agreement (if applicable), confidential subject identification code list form, drug accountability forms, investigator and study staff list form.

20.2 Protocol amendments

In order to obtain interpretable results, neither the investigator nor the sponsor will alter the study conditions agreed upon and set out in this protocol. Amendments should be made by mutual agreement between the investigator and the sponsor. Any amendment must be set out in writing, giving the reasons, and being signed by all concerned parties. The amendment becomes then part of the protocol.

20.3 Study documentation and record keeping

The investigator should ensure the accuracy, completeness, legibility, and timeliness of the data reported to the sponsor in the CRFs and in all required reports.

The investigator must keep source documents for each subject in the study. All information on the CRFs must be traceable to these source documents, which are generally stored in the subject's medical file. The source documents should contain all demographic and medical information, including laboratory data, ECGs, etc., and the original signed informed consent forms.

Data reported on the CRF that are derived from source documents should be consistent with the source documents or the discrepancies should be explained.

The investigator and the sponsor should maintain the study documents as specified in the "Essential Documents for the Conduct of a Clinical Trial" chapter 8 of ICH-GCP and as required by CFDA GCP and the applicable regulatory requirement(s).

These are documents which individually and collectively permit evaluation of a study and the quality of the data produced and include groups of documents, generated before the study commences, during the clinical study, and after termination of the study and include but are not limited to, study protocol, amendments, submission and approval of EC, raw data of subjects including lab tests and ECG tracing, insurance contracts, certificate of analysis of the IMP(s),

drug accountability records, signed informed consent forms, confidential subjects identification code, CRFs, curricula vitae of the investigator and other participants in the study, study staff lists and responsibilities, monitoring reports and final study report.

The investigator and the sponsor should take measures to prevent accidental or premature destruction of these documents.

Study documents must be retained by the investigator and the sponsor as long as needed to comply with ICH-GCP, CFDA GCP, national and international regulations. By signing the protocol, the investigator and the sponsor agree to adhere to these requirements.

20.4 Study subjects' recruitment

Study participants will be recruited from the volunteers' database. The study site may also use the site management organisation to recruit the study participants.

In addition, they may also use recruitment posters in other departments within the hospital, community outside or newspapers to raise awareness of the study that is going on.

Other departments within the hospital will be involved to recommend study participants as well.

The clinical site has detailed SOPs on the recruitment process.

20.5 Confidentiality and data protection

By signing this protocol, the investigator and the CRO agree to keep all the information provided by the sponsor in strict confidentiality and to request the same confidentiality from his/her staff. Study documents provided by the sponsor (protocols, IB, CRFs and other materials) will be stored appropriately to ensure confidentiality. The information provided by the sponsor to the investigator and to the CRO cannot be disclosed to others without direct written authorisation from the sponsor, except for the extent necessary to obtain the informed consent from the subjects wishing to participate in the study.

Data on subjects collected in the CRFs during the study will be documented in an anonymous way. If, as an exception, for safety or regulatory reasons identification of a subject becomes necessary, the monitor, the sponsor and the investigator will be bound to keep this information confidential.

20.6 Publication policy

The sponsor agrees that the study results (including negative and inconclusive as well as positive results) can be made publicly available by the investigator publishing in peer reviewed journals, presenting results at scientific congresses and posting information and results on internet-based public registers and databases.
Study results will be communicated in full to the competent Health Authorities by the submission of a complete clinical study report.

As the sponsor agrees that the study results can be published by the investigator(s), the investigator agrees to submit any manuscript (abstract, publication, paper, etc.) to the sponsor before any public disclosure.
This will be done in order to ensure that clinical study results are reported in an objective, accurate and balanced manner. The sponsor reviews the proposed manuscripts, before submission, within a reasonable period of time (30-90 days in relation with the complexity of the work).

The investigator will also be provided by the sponsor with the clinical study report and the results of any additional analysis, tables, figures, etc. undertaken for the purposes of the article, in order to take responsibility for the content of the publication(s).
On an exceptional basis, the sponsor may temporarily delay registration of certain data elements (e.g. compound, name, outcome, measures, etc.) to seek necessary intellectual property protection. This is because early disclosure of such data could, in some circumstances, prevent or negatively impact patentability.

21 STUDY RESPONSIBLE PERSONS

21.1 Sponsor

Zambon S.p.A., via Lillo del Duca 10, I-20091 Bresso, Italy
Phone: +39.02.66.52.41
Fax: +39.02.66.50.14.92

Protocol Review Committee Chairman
PPD, Global Chief Medical Officer and Patient's Access Head

Sponsor's representatives
PPD, Global Medical Affairs Head Established and Respiratory Medicine

21.2 Drug assay

Eurofins Central Laboratory Shanghai, 395 Jiang Chang West Road, 7th Floor, Shanghai, 200436 China

Analytical facility:
United-Power Pharma Tech Co., Ltd. (UP-Pharma)
2F, Tower B, No. 33 Science Park Road,
Changping District, Beijing, P.R.China
ZIP Code: 102206

21.3 Project management, data analysis & reporting, monitoring

PAREXEL International (IRL) Limited ("**PAREXEL**"), a corporation organized under the laws of Ireland with a registered address at 70 Sir John Rogerson's Quay, Dublin 2, Ireland.

22 REFERENCES

1. Ventresca GP, Cicchetti V, Ferrari V. Drugs in Bronchial Mucology. 1989. Cap. 4.1 Thiols 77-102. Edited by PC Braga and Allegra. Raven Press Ltd
2. Aruoma OI, Halliwell B, Hoey BM, Butler J. The antioxidant action of N-acetylcysteine: Its reaction with hydrogen peroxide, hydroxyl radical, superoxide and hypochlorous acid. Free Radical Biol Med 1988; 6:593-597
3. Ziment I. Acetylcysteine: a drug that is much more than a mucokinetic. Biomed & Pharmacother 1988; 42: 513-520
4. Borgstrom L, Kagedal B, Paulsen O. Pharmacokinetics of N-acetylcysteine in man. Eur J Clin Pharmacol 1986; 31: 217-222
5. Olsson B, Johansson M, Gabrielsson J, Bolme P. Pharmacokinetics and bioavailability of reduced and oxidized N-acetylcysteine. Eur J Clin Pharmacol 1988; 34: 77-82
6. Burgunder JM, Varriale A, Lauterburg BH. Effect of N-acetylcysteine on plasma cysteine and glutathione following paracetamol administration. Eur J Clin Pharmacol 1989; 36: 127-131
7. De Caro L, Ghizzi A, Costa R, Longo A, Ventresca GP, Lodola L. Pharmacokinetics and bioavailability of oral acetylcysteine in healthy volunteers. Arzneim-Forsh/Drug Res 1989; 39: 382-386
8. Borgstrom L, Kagedal B. Dose dependent pharmacokinetics of N-acetylcysteine after oral dosing to man. Biopharmaceutics & Drug Disposition 1990; 11: 131-136
9. Frascio M. Pharmacokinetic study after single dose (1, 2, and 4 g) of a new N-acetylcysteine (NAC) oral formulation. Zambon Group Internal Report, code ZPM/94/34
10. Crestani S. Open label, randomized, cross-over, pharmacokinetic study to assess the bioequivalence of two oral N-Acetylcysteine preparations: 600 mg film coated tablets versus 600 mg effervescent tablets. 2002 Report in Zambon Group's file
11. Rusca A. Study CRO-PK-14-286 – Sponsor code Z7169J02. Comparative bioavailability study of N-acetyl-cysteine (NAC) 600 mg uncoated tablets vs. NAC 600 mg film-coated tablets in healthy male and female volunteers. CROSS Research S.A., Switzerland 2014
12. Rusca A. Study CRO-PK-07-189 – Sponsor code 7169J01. Comparative bioavailability study of a new oral N-acetyl-cysteine (NAC) formulation vs. Fluimucil®, Zambon in healthy volunteers. CROSS Research S.A., Switzerland, 2008
13. Rusca A. Study CRO-PK-09-225 – Sponsor code 7203J01. Pilot bioavailability study of a new 1200 mg N-acetyl-cysteine (NAC) effervescent tablet formulation vs. Fluimucil® 600 mg effervescent tablet (Zambon S.p.A., Italy) in healthy male and female volunteers. CROSS Research S.A., Switzerland, 2010
14. Brown M, Bjorksten A, Medved I and M McKenna. Pharmacokinetics of intravenous N-acetylcysteine in men at rest and during exercise. Eur J Clin Pharmacol 2004;60:717-723
15. Jones AL, Jarvier D. Simpson D. Pharmacokinetics of N-acetylcysteine are altered in patients with chronic liver diseases. Aliment Pharmacol Ther. 1997; 787-791
16. Prescott LF, Donovan JW, Jarvie DR, Proudfoot AT. The disposition and kinetics of intravenous N-acetylcysteine in patients with paracetamol overdosage. Eur J Clin Pharmacol 1989; 37: 501-506
17. Pendyala L, Creaven PJ. Pharmacokinetic and pharmacodynamic studies of N-acetylcysteine, a potential chemopreventing agent during a phase I trial. Cancer Epidemiology, Biomarkers and Prevention 1995; 4: 245-251
18. Rodenstein D, De Coster A, Gazzaniga A. Pharmacokinetics of oral acetylcysteine: absorption, binding and metabolism in patients with respiratory disorders. Clinical Pharmacokinetics 1978; 3: 247-254
19. Pharmacokinetics of N-Acetylcysteine following repeated i.v. infusions to hemodialysed chronic renal failure patients. Internal Report 2002. Zambon Group's file

20. Sjodin K, Nilsson E, Hallemberg A, Tunek A. Metabolism of N-acetyl-l-cysteine Biochem. Pharmacol 38 1989; 22: 3981-3985
21. Locatelli C. Fluimucil 20% injectable (N-Acetylcysteine). Expert Report on the clinical documentation 1996. Zambon Group's file
22. N-acetyl-L-cysteine (NAC) intravenous Investigator's Brochure, edition number 1, 20 April 2018
23. Guidelines on GMP, published by the Commission in The rules governing medicinal products in the European Community, Volume 4
24. Guidance on the investigation of bioequivalence. CPMP/EWP/QWP/1401/98 Rev. 1/Corr **, 20 January 2010
25. U.S. Department of Health and Human Services and U.S. Department of Agriculture, Nutrition and your health: 2015-2020 Dietary Guidelines, Appendix 9