- **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:** 4.0
- **Date of the document:** 15 June 2020

16.1.9 Documentation of Statistical Methods

The following documents are included:

- Statistical Analysis Plan, Version 4.0, dated 15 Jun 2020

[redacted]

Previous versions of Statistical Analysis Plan (Version 1.0, dated 18 Apr 2019, Version 2.0, dated 04 Nov 2019, and Version 3.0, dated 21 Apr 2020) and Statistical Analysis Report (Version 1.0, dated 10 Jul 2020) are in [redacted] and are available upon request.

PAREXEL International

Zambon S.p.A.

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

Statistical Analysis Plan

PAREXEL Project Number: CCI

SPONSOR SIGNATURE PAGE

Approved by: PPD

and Patient's Access Head
Zambon S.p.A.

PAREXEL SIGNATURE PAGE

Signatures below confirm that the review process has been completed in accordance with [redacted]

This document has been approved and signed electronically on the final page by the following:

Signatory	
Author	PPD Project Role: PPD

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REVISION HISTORY

Version No.	Effective Date	Summary of Change(s)
Draft 0.1	29 Aug 2018	New document
Draft 0.2	01 Mar 2019	Revised from draft version for CTA submission (draft 0.1), based on final protocol 2.0, 19 Dec 2018.
Draft 0.3	12 Mar 2019	Revised based on internal review.
Draft 0.4	08 Apr 2019	Revised based on sponsor review and internal review on TLF Shells: 1) Move screening lab tests analysis from Safety section to Demographic and Baseline section. 2) Add shifting table to present change from baseline for categorical safety evaluations (clinically significance and CTCAE Grade). 3) Add summary of cumulative urine PK concentration.
Final 1.0	18 Apr 2019	Upgraded version to Final 1.0.
Draft 1.1	28 Oct 2019	1) Update urinalysis parameters in section 3.2.2 Safety Variables according to protocol v2.0 memorandum. 2) Update section 4.7: concomitant procedures are analyzed in Safety set. 3) Update section 4.10.1: The summary of adverse events is analyzed by period as well.
Draft 1.2	30 Oct 2019	1) Adding PK relevant plots during multiple dose. 2) Remove physical examination on Day 1. 3) Alcohol breath test is added in section 4.5. 4) The surgical history is added in section 4.6.
2.0	04 Nov 2019	Upgraded version to Final 2.0.
Draft 2.1	07 Feb 2020	The major change includes following: 1. Section 4.7: Updated WHODrug and MedDRA version. 2. Section 4.9.3: removed "all other descriptive statistics will be reported to three significant digits" to show more significant digits in AUC_{min} and AUC_{max}; updated statistical outlier testing rule in PK concentration data. 3. Section 4.10.1: Updated MedDRA version; updated "Any TEAE leading to dose reduced" as "Any TEAE leading to dose interruption". 4. APPENDIX 3: Remove duplicate rule to grade Hyper Serum creatinine based on BL (baseline) value; deleted coagulation.
3.0	21 Apr 2020	Upgraded version to Final 3.0.

3.1	12 Jun 2020	After database locked, SAP appendix updated to be clearer after final TFL output reviewed by Sponsor: 1. APPENDIX 2 Partial Date Conventions: The imputation rule of partial date for dates of ECG, laboratory data, standardized meals, physical examination, substance usage and vital sign is specified clearly in Appendix. This update in SAP doesn't affect final TFL results, just to ensure consistency in ADaM spec derivation, datasets programming and final TFL outputs. 2. APPENDIX 3 CTC Grading for Laboratory Parameters: update wording of LLC as LLN and ULC as ULN, to ensure consistency between SAP and final TFL outputs. Also updated in List of Abbreviation. 3. PK results of BLQ is corrected as BQL to ensure consistency between SAP and outputs. Also updated in List of Abbreviation.
4.0	15 Jun 2020	Upgraded version to Final 4.0.

LIST OF ABBREVIATIONS

Abbreviation / Acronym	Definition / Expansion
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
AST	Aspartate aminotransferase
$AUC_{(0-t)}$	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 from single dose to 12h postdose
$AUCss_{(0-t)}$	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
BQL	Below Quantification Limit
BMI	Body Mass Index
BP	Blood Pressure
C_{max}	Peak drug concentration
CLt	Total body clearance
CLr	Renal clearance
CRF	Case Report Form
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
CTCAE	Common Terminology Criteria for Adverse Events
CV	Coefficient of Variation
DBL	Database Lock
DBP	Diastolic Blood Pressure
DF	Degree of fluctuation
DTT	Dithiothreitol
ECG	Electrocardiogram
ETV	Early Termination Visit
Fe_{0-t}	Total fraction of NAC dose excreted in urine
F_{rel}	Relative Bioavailability
HBs Ag	Hepatitis B virus surface antigen
HCV Ab	Hepatitis C virus antibodies
HIV	Human Immunodeficiency Virus
HR	Heart Rate
ICH	International Conference on Harmonisation
i.m.	Intramuscular

IMP	Investigational Medicinal Product
i.v.	Intravenous
k_{el}	Terminal elimination rate constant
LQL	Lower Quantification Limit
LLN	Lower limit of Normal
MCH	Mean Cell Haemoglobin
MCHC	Mean Cell Haemoglobin Concentration
MCV	Mean Cell Volume
MedDRA	Medical Dictionary for Regulatory Activities
NAC	N-acetyl-L-cysteine
NC	Not calculated
NCI	National Cancer Institute
NCS	Not clinically significant
PK	Pharmacokinetics
PT	Preferred Term
PTAE	Pre-Treatment Adverse Event
R	Accumulation ratio
RBC	Red Blood Cells
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SBP	Systolic Blood Pressure
SD	Standard Deviation
SOC	System Organ Class
SOP	Standard Operating Procedure
SDTM	Study Data Tabulation Model
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}
ULN	Upper Limit of Normal
Vd	Volume of distribution
WBC	White Blood Cells

1 INTRODUCTION

This SAP is based upon the following study documents:

- Study Protocol, Version 3.0 (Dec 03, 2019)
- electronic Case Report Form (eCRF), Version 3.0 (Oct 14, 2019)

The Statistical Analysis Plan (SAP) details the statistical methodology to be used in analyzing study data and outlines the statistical programming specifications for the Tables, Listings, and Figures. It describes the variables and populations, anticipated data transformations and manipulations and other details of the analyses not provided in the Clinical Study Protocol (CSP).

The SAP will be finalized prior to database lock (DBL) and describes the statistical analysis as it is foreseen when the study is being planned. If circumstances should arise during the study rendering this analysis inappropriate, or if improved methods of analysis should arise, updates to the analyses may be made. Any deviations from the SAP after DBL, reasons for such deviations and all alternative or additional statistical analyses that may be performed, will be described in a SAP Addendum.

2 STUDY OBJECTIVES

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

2.1 Primary End-Points

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

2.2 Secondary End-Points

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

3 INVESTIGATIONAL PLAN

3.1 Overall Study Design and Plan

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. Study schedule is described as Appendix 1.

3.2 Pharmacokinetics and Safety Variables

3.2.1 Pharmacokinetics Variables

The derivation of PK parameters will be the responsibility of Quantitative Clinical Development (QCD), PAREXEL International. PK parameters will be calculated by non-compartmental analysis methods following these guidelines;

- Actual sampling times relative to dosing rather than nominal times will be used in the calculation of all derived PK parameters.
- There will be no imputation of missing data.
- Any subjects with missing concentration data will be included in the analysis provided that at least C_{max} and $AUC_{(0-t)}$ can be reliably calculated

The following PK parameters will be measured and/or calculated for plasma and urine NAC data, using the validated software Phoenix® WinNonlin® version 8.0 or higher (Certara USA, Inc., Princeton, NJ) (actual version will be stated in the final report), after a single dose of the Investigational Medicinal Product (IMP):

C_{max}: Maximum NAC plasma concentration will be obtained directly from the concentration data

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

k_{el}: Terminal elimination rate constant, calculated, by linear regression s
- Only those data points that are judged to describe the terminal log-linear decline will be used in the regression.
- A minimum number of three data points in the terminal phase will be used in calculating Kel with the line of regression starting at any post-C_{max} data point (C_{max} should not be part of the regression slope) and including C_{last}, t_{last}.
- The correlation coefficient (R^2) in general should be greater than 0.80.

$t_{1/2}$: 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 from single dose to 12 h post-dose, calculated with the linear up/log down trapezoidal method

$\%AUC_{extra}$: Percentage of the $AUC_{(0-\infty)}$ obtained by extrapolation, calculated as; $(1 - [AUC_{(0-t)}/AUC_{(0-\infty)}])\times 100$

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 $Dose/ AUC_{(0-\infty)}$

V_{ur}: Measured overall volume of urine collected during each collection interval.

$Ae_{(t1-t2)}$: Amount excreted in the urine at each interval collection

$Ae_{(0-tz)}$: Cumulative amount excreted up to tz hours, where tz is 4, 8, 12, 24 or 32 will be calculated as the sum of the products of concentration and urine volume over the appropriate collection intervals

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

$Fe_{(0-tz)}$: The fraction of unchanged drug excreted in urine will be calculated as fe=Ae/Dose, up to tz hours, where tz is 4, 8, 12, 24 or 32 hours

$Fe_{(0-t)}$: 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, after multiple doses of IMP:

C_{ss_max}: Maximum NAC plasma concentration at steady-state will be obtained directly from the concentration data

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

C_{ss_min}: Trough NAC plasma concentration at steady-state, measured as the 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).

BQL values will be imputed in the PK concentration dataset used for the derivation of PK parameters. The following rules will be applied:

- BQLs at the beginning of a subject profile (i.e., before the first incidence of a measurable concentration) will be assigned to zero.
- BQLs at the end of a subject profile (i.e., after the last incidence of a measurable concentration) will be set to missing.
- Single BQLs which fall between two measurable concentrations and consecutive BQLs which fall between measurable concentrations will be set to missing.

3.2.2 Safety Variables

Safety and general tolerability of the IMP variables include:

- Treatment-Emergent Adverse Event (TEAEs)
- Physical examinations including body weight at screening, day 3 and final visit/ETV.
- Vital signs at screening, day 1 (pre and post dose), day 3 (post dose), day 6 (pre and post dose) and Final visit (post dose)/ETV.
- 12-lead ECG at screening.

- Laboratory tests at screening, day 3 and the final visit/ETV:
  - 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.
  - Urine analysis:
    Urine chemical analysis: pH, specific gravity, appearance, colour, nitrites, proteins, glucose, urobilinogen, bilirubin, ketones, blood, leukocytes, erythrocytes, epithelial, crystals, cylinders.

4 STATISTICAL METHODS

4.1 Data Quality Assurance

All tables, figures and data listings to be included in the report will be independently checked for consistency, integrity and in accordance with standard PAREXEL procedures.

4.2 General Presentation Considerations

'Baseline' is defined as the last available pre-treatment assessment. 'End of Study' is defined as the last available post-treatment assessment. 'Treatment Day' will be calculated relative to the date of first dose i.e. Treatment Day = Assessment Date -First Dose Date + 1.

Continuous data will be summarized in terms of the mean, standard deviation (SD), median, minimum, maximum and number of observations, unless otherwise stated. Continuous data that are expected to be skewed will be presented in terms of the maximum, upper quartile, median, lower quartile, minimum and number of observations. The minimum and maximum will be reported to the same number of decimal places as the raw data recorded in the database. The mean, median, lower quartile and upper quartile will be reported to one more decimal place than the raw data recorded in the database. The SD will be reported to two more decimal places than the raw data recorded in the database. In general, the maximum number of decimal places reported shall be four for any summary statistic.

Categorical data will be summarized in terms of the number of subjects providing data at the relevant time point (n), frequency counts and percentages. Any planned collapsing of categories will be detailed in the SAP text and the data displays.

Percentages will be presented to one decimal place. Percentages will not be presented for zero counts. Percentages will be calculated using n as the denominator. If sample sizes are small, the data displays will show the percentages, but any textual report will describe frequencies only.

Changes from baseline in categorical data will be summarized using shift tables where appropriate.

P-values greater than or equal to 0.001, in general, will be presented to three decimal places. P-values less than 0.001 will be presented as "<0.001".

Confidence intervals will be presented to one more decimal place than the raw data.

No available data will be evaluated as "missing values".

All report outputs will be produced using SAS® version [9.4] or a later version in a secure and validated environment. All report outputs will be provided to the Sponsor in a single Microsoft Word document.

4.3 Study Subjects

4.3.1 Disposition of Subjects

Subject disposition will be listed and summarized. The summary will include the number and percentage of subjects:

- Screened
- Screen failure and primary reason
- Treated
- Discontinued treatment and primary reason
- Discontinued the study and primary reason

4.3.2 Protocol Deviations

Major protocol deviations and any action to be taken regarding the exclusion of subjects or affected data from PK set are defined in the project-specific Protocol Deviation Specification.

- A summary of the number and percentage of subjects with a major protocol deviation by type of deviation will be provided for Enrolled set.

- A summary of the number and percentage of subjects excluded from PK set by type of reason will be provided for Enrolled set.

- A by-subject listing of protocol deviations will be provided.

4.4 Analysis Populations

4.4.1 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.

4.4.2 Reasons for exclusion from the PK set before statistical analysis

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
- Adverse events (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 Case report form (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 $AUC_{0-\infty}$.

4.4.3 Analysis

- A summary of subjects included in each analysis set will be provided for Enrolled Set.
- By-subject listing including analysis set flag (Yes or No), and reason of exclusion from safety and PK set will be provided.

4.5 Demographic and Other Baseline Characteristics

The following summaries will be provided for demographic and other baseline characteristics based on Enrolled set.

- A summary of demographic variables and baseline characteristics:
  Age (years), sex, ethnicity, height (cm), weight (kg), BMI (kg/m^2).
  BMI (kg/m^2) = weight (kg) / [height (cm)]^2

- A summary of other possibly relevant variables:
  Alcohol consumption, tobacco consumption, caffeine consumption, and history of drugs.

- By-subject listings of demographic data and other baseline characteristics (as summarized above) will be provided.

Other lab tests performed during screening and baseline visits will also be listed:

- Serum virology and bacteriology at screening: Hepatitis B (HBs antigen), Hepatitis C (HCV antibodies), HIV 1/2 (HIV Ag/Ab combo), Treponema pallidum.
- Urine drug test at screening and Visit 2, day -1: cocaine, amphetamine, methamphetamine, cannabinoids (delta-9-tetrahydrocannabinol - THC), opiates
- Alcohol breath test.
- Serum pregnancy test at screening.
- Urine pregnancy test at Visit 2, day -1.

4.6 Medical and Surgical History

Medical and surgical history will be coded using the Medical Dictionary for Regulatory Activities (MedDRA version 22.0).

A summary of medical and surgical history by system organ class (SOC) and preferred term (PT) on Enrolled Set will be provided.

By-subject listings of medical and surgical history will also be provided.

4.7 Prior and Concomitant Medications/Procedures

Prior and Concomitant medications will be coded using the WHO Drug Dictionary Enhanced (WHODrug Global version March 2019).

Prior and Concomitant procedures will be coded using the Medical Dictionary for Regulatory Activities (MedDRA version 22.0).

Medications will be classified as "Prior" or "Concomitant" based on the comparison between start and stop dates of the medication and the date of first dose of study drug.

Medications that stop prior to the date of first dose of study drug will be classified as "Prior Medications".

Medications that stop on or after the date of first dose of study drug will be classified as "Concomitant Medications". Imputation of partial dates of medication records for classification of "prior" and "concomitant" is described in Appendix 2.

Prior and concomitant procedures will be classified in the same way as medications.

- A summary of concomitant medications by ATC level 3 term and preferred name will be provided for Enrolled Set.
- A summary of concomitant procedures by SOC and PT will be provided for Safety Set.
- By-subject listing of concomitant medications and procedures will be provided.
- By-subject listing of prior medications and procedures will be provided.

4.8 Treatment Compliance

Exposure and compliance data will be summarized and listed based on Safety Set.

- A descriptive summary of treatment compliance variables will be provided:
    - Treatment duration (days) = last dose date – first dose date + 1
    - Total amount of exposure doses (ml)
    - Treatment compliance (%) = (total amount of actual dosage infused/total amount of prescribed dosage)*100
    - Treatment compliance by categories (<80%, 80%-120%, >120%)

Detailed IMP administration data and the treatment compliance variables will be listed.

Detailed standardized meals will also be listed.

4.9 Pharmacokinetics Analysis

4.9.1 Pharmacokinetic Concentrations

Pharmacokinetic plasma and urine concentration data for NAC will be listed by subject. Listings will include actual sampling times relative to dose administration, in addition the urine data listing will include the volume of urine collected, the concentration of NAC and the amount of NAC excreted per collection interval.

Plasma concentrations and urine amounts of NAC will be summarized descriptively. The following descriptive statistics will be presented for plasma and urine data obtained at each

nominal time point: n, arithmetic mean, SD, coefficient of variation (CV%), geometric mean, geometric SD, geometric CV% (calculated as: gCV% =SQRT(e^{s^2}-1)*100; where s is the standard deviation of the log-transformed values), median, minimum and maximum values.

The following rules will be followed with regards to the number of decimal places and presentation of data in the tables and listings of concentration data:

- Source data shall be used in all derived PK concentrations without prior rounding
- The mean, standard deviation (SD), geometric mean and median will be tabulated to one more significant digit compared to the source data, but with a maximum of four significant digits.
- Minimum and maximum values will be tabulated to the same precision as the source data, but with a maximum of four significant digits.
- Geometric coefficient of variation (CV) % and coefficient of variation (CV%) will be presented to one decimal place.

The following descriptive pharmacokinetic graphs will be generated:

- Subject Profiles for NAC Plasma Concentration vs. Time Data for Single Dose – Linear Scale
- Subject Profiles for NAC Plasma Concentration vs. Time Data for Multiple Dose – Linear Scale
- Subject Profiles for NAC Plasma Concentration vs. Time Data for Single Dose – Semi-Logarithmic Scale
- Subject Profiles for NAC Plasma Concentration vs. Time Data for Multiple Dose – Semi- Logarithmic Scale
- Arithmetic Mean (± SD) NAC Plasma Concentration vs. Time Data for Single Dose – Linear Scale
- Arithmetic Mean (± SD) NAC Plasma Concentration vs. Time Data for Multiple Dose – Linear Scale
- Arithmetic Mean NAC Plasma Concentration vs. Time Data for Single Dose – Semi-Logarithmic Scale
- Arithmetic Mean NAC Plasma Concentration vs. Time Data for Multiple Dose – Semi-Logarithmic Scale
- Subject Profiles for Cumulative NAC Urine Amount Excreted vs. Time Data for Single Dose – Linear Scale
- Subject Profiles for Cumulative NAC Urine Amount Excreted vs. Time Data for Multiple Dose – Linear Scale
- Subject Profiles for Cumulative NAC Urine Amount Excreted vs. Time Data for Single Dose – Semi-Logarithmic Scale
- Subject Profiles for Cumulative NAC Urine Amount Excreted vs. Time Data for Multiple Dose – Semi-Logarithmic Scale

- Arithmetic Mean (± SD) Cumulative NAC Urine Amount Excreted vs. Time Data for Single Dose – Linear Scale
- Arithmetic Mean (± SD) Cumulative NAC Urine Amount Excreted vs. Time Data for Multiple Dose – Linear Scale
- Arithmetic Mean Cumulative NAC Urine Amount Excreted vs. Time Data for Single Dose – Semi-Logarithmic Scale
- Arithmetic Mean Cumulative NAC Urine Amount Excreted vs. Time Data for Multiple Dose – Semi-Logarithmic Scale

4.9.2 Handling of Values Below the Limit of Qualification (BQL)

For listings, all concentrations below the limit of quantification (BQL) will be labeled as "BQL" in the concentration data listings. Missing data will not be imputed.

For calculation of descriptive statistics of concentration by time point and graphs of arithmetic means, values that are BQL will be substituted with zero.

For calculation of geometric means, as a consequence of BQL values, calculated geometric means could be null. In the presence of any null values, the geometric mean will be reported as not calculated (NC).

4.9.3 Pharmacokinetic Parameters

Pharmacokinetic parameters will be listed by subject. Descriptive statistics for calculated PK parameters will include: n, arithmetic mean, SD, CV%, geometric mean, geometric CV%, median, minimum and maximum values. For t_{max}, only median, minimum and maximum values will be presented. No descriptive statistics will be determined when fewer than three individual PK parameters are available.

The following rules will be followed with regards to the number of decimal places and presentation of data in the tables and listings for PK parameters:

- Individual PK parameters will be presented to four significant digits, with the exception of t_{max}, which will be presented to two decimal places.
- Parameters derived directly from source data (e.g. C_{max},) shall be reported with the same precision as the source data (if this is not four significant digits).
- The mean, geometric mean, median and SD values will be reported to four significant digits except for CV% which will be presented to one decimal place.
- For t_{max} the minimum and maximum will be presented to two decimals places and all other descriptive statistics will be presented to three decimal places.
- Estimates and confidence intervals in the form of percentages will be presented to two decimal places.

After receiving Plasma and urine PK concentration data from Vendor, statistical outlier testing will be performed by using boxplots to identify outliers as described below:

- Q1: 25th percentiles; Q3: 75th percentiles; Interquartile Range (IQR) = Q3 – Q1;
- Lower Far outlier: value < Q1 – 3 × IQR;
- Upper Far outlier: value < Q3 + 3 × IQR.

The far outliers will be identified, reviewed and discussed during the Data Review Meeting prior to Database lock. Outliers that may have an impact on the concentration-time profiles will be discussed and it will be determined whether to exclude them from PK concentrations, PK parameters or PK set. As a general rule, outliers will be ruled out from tabulations but kept in data listings.

If a subject or outlier is required to be excluded from PK analysis, details will be documented in Data Review Meeting minutes, including an external excel file that documents the required Analysis Flag on applicable analysis datasets level.

4.10 Safety Evaluation

All safety summaries and analyses will be based upon the Safety Set.

4.10.1 Adverse Events

AEs will be coded by System Organ Class (SOC) and Preferred Term (PT), using the Medical Dictionary for Regulatory Activities (MedDRA version 22.0).
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

Where dates are missing or partially missing, adverse events will be assumed to be treatment-emergent, unless there is clear evidence (through comparison of partial dates) to suggest that the adverse event started prior to the first dose of IMP. The detailed rules are described in Appendix 2.

Individual PTAEs and TEAEs will be listed in subject data listings. No summary table will be provided for PTAEs.

Serious adverse event (SAEs) occurring or worsening after the first dose of IMP are defined as TESAEs.

For all TEAE tables, a subject will be counted only once for each System Organ Class (SOC) and each PT, even if the subject reported more than one event under each subcategory. Unless specified otherwise, TEAE tables will be ordered in terms of

decreasing number of subjects for SOC and then PT within the SOC in the column of "NAC 600 mg", and then alphabetically for SOC and PT within the SOC if the frequency is tied.

For each subject and each adverse event, the worst severity recorded will be attributed and used in the by-severity summaries. Similarly, the worst causality (most related to treatment) will be attributed and used in the by-causality summaries. If severity or causality is missing, the worst case will be assumed.

An overview table will summarize the number and percentage on subject level of the following categories and by period (single and multiple dose):

- Any TEAE
- Any TEAE related to IMP
- Any severe TEAE
- Any severe TEAE, related to IMP
- Any TEAE with outcome of death
- Any TEAE with outcome of death, related to IMP
- Any TESAE
- Any TESAE, related to IMP
- Any TEAE leading to discontinuation of IMP
- Any TESAE leading to discontinuation of IMP
- Any TEAE leading to discontinuation of IMP, related to IMP
- Any TEAE leading to dose interruption

TEAE summaries will present the number and percentage of subjects reporting at least one TEAE. The following summaries will be provided:

- TEAEs by SOC and PT
- TEAEs by SOC and PT, by maximum severity
- TEAEs by SOC and PT, by maximum relationship
- TEAEs related to IMP, by SOC and PT
- TESAEs by SOC and PT
- TESAEs by SOC and PT, by maximum severity
- TESAEs by SOC and PT, by maximum relationship
- TESAEs related to IMP, by SOC, PT
- TEAE leading to IMP permanently stopped, by SOC and PT
- TEAE leading to death, by SOC and PT

The number and percentage of deaths will also be summarized.

The following by-subject AE listings will be provided:

- TEAEs
- TESAEs
- TEAEs related to IMP
- TEAEs leading to drug permanently stopped
- TEAEs leading to death
- PTAEs
- Deaths

The listings will include: subject identifier, age, sex, race, adverse event (SOC, PT, and verbatim term), period, date of onset, date of resolution, duration, severity, seriousness, action taken, outcome and causality.

4.10.2 Clinical Laboratory Evaluation

Laboratory values (hematology, biochemistry, and urinalysis) will be listed by subject and study time point including changes from baseline for continuous variables. The baseline for the laboratory values will be the results obtained on the last measurement before the first dose.

All values outside the clinical reference ranges will be flagged in the data listings. The abnormal values will be flagged with 'L' for values below the lower limit of the clinical reference range and 'H' for values above the upper limit of the clinical reference range and included in the listings. The Investigator will assess whether the values outside the clinical reference range are clinically significant and these will be reported as "abnormal, not clinically significant (NCS)" or "abnormal, clinically significant (CS)". Clinically significant laboratory values will be recorded by the Investigator as AEs.

Quantitative laboratory assessment reported as "<X", i.e. BQL, or ">X", i.e. above the upper limit of quantification, will be converted to X for the purpose of quantitative summaries, but will be presented as recorded, i.e. as "<X" or ">X" in the listings.

The toxicity grading of laboratory tests is determined based on the NCI CTCAE V4.03 (Appendix 3).

The following summaries will be provided for laboratory data:

- Absolute value and change from baseline for continuous data by scheduled time point.
- The number and percentage of subjects in each test result category for categorical data, by scheduled time point.
- Shifting from baseline of subjects with lab results evaluation as Normal, NCS, and CS, by scheduled time point.
- Shifting from baseline of subjects with lab results evaluated by NCI CTCAE V4.03 by toxicity grading and scheduled time point.

The following laboratory data listings will be provided:

- Listing of laboratory test results
- Listing of abnormal laboratory test results
- Listing of other lab tests and pregnancy tests.

4.10.3 Vital Signs, body weight, 12-Lead Electrocardiogram (ECG), and Physical Examinations

The following summaries and listings will be provided for vital signs and body weight:

- Absolute value and change from baseline of vital signs parameters and body weight with descriptive statistics, by scheduled time point.
- Listing of vital signs and body weight data.

12-Lead ECG will be performed at screening only and will not be summarized. A listing will be provided for ECG performance and interpretation.

Physical examinations will not be summarized. A listing of physical examination data will be provided with investigator's interpretation.

4.11 Determination of Sample Size

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.

4.12 Changes in the Conduct of the Study or Planned Analysis

Not Applicable.

5 REFERENCES

No Reference.

6 APPENDIX

APPENDIX 1 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*

APPENDIX 2 Partial Date Conventions

Imputed dates will NOT be presented in the listings. However, in general, when calculating relative days, partial dates of ECG, laboratory data, standardized meals, physical examination, substance usage and vital sign with missing day only will be assumed to be 15th of the month, and partial dates with both missing day and month will be assumed to be June 30.

Otherwise, for partial dates of medical history, concomitant medication and procedures the following rules in the given table will be applied for each case.

Algorithm for Medical History, Prior/Concomitant Medications, Therapies or Procedures:

Start Date	Stop Date	Action
Known	Known	If stop date is prior to the date of first dose of study drug, considered as prior; if start date is on or after the date of first dose of study drug, considered as concomitant.
	Partial	The last day of the month and the last month (i.e. December) will be used if the day/month of stop date is missing. If the impuated stop date is prior to the date of first dose of study drug, considered as prior; if the imputed stop date is on or after the date of first dose of study drug, considered as concomitant.
	Missing	Considered as concomitant.
Partial	Known	The first day of the month and January will be used if the start day/month is missing. If stop date is prior to the date of first dose of study drug, considered as prior; if the stop date is on or after the date of first dose of study drug, considered as concomitant.
	Partial	The first day of the month and January will be used if the start day/month is missing. The last day of the month and the last month (i.e. December) will be used if the day/month of stop date is missing. If the imputed stop date is prior to the date of first dose of study drug, considered as prior; if the imputed stop date is on or after the date of first dose of study drug, considered as concomitant.
	Missing	The first day of the month and January will be used if the start day/month is missing. Considered as concomitant.

Start Date	Stop Date	Action
Missing	Known	If stop date is prior to the date of first dose of study drug, considered as prior; if stop date is on or after the date of first dose of study drug, considered as concomitant;
	Partial	The last day of the month and the last month (i.e. December) will be used if the day/month of stop date is missing. If the impuated stop date is prior to the date of first dose of study drug, considered as prior; if the imputed stop date is on or after the date of first dose of study drug, considered as concomitant.
	Missing	Considered as concomitant.

Algorithm for Treatment-Emergent Adverse Events:

Start/ Increase Severity Date	Stop Date	Action
Known	Known	Considered as a TEAE if start/increase severity date on or after the date of the first dose of study drug.
	Partial	Considered as a TEAE if start/increase severity date on or after the date of the first dose of study drug and on or before the last dose of study drug + 30 days. The last day of the month and the last month (i.e. December) will be used if the stop day/month is missing.
	Missing	Considered as a TEAE if start/increase severity date on or after the date of the first dose of study drug and on or before the last dose of study drug + 30 days.
Partial, but known components show that it cannot be on or after first study drug taken date.	Known	Not a TEAE. The first day of the month and January will be used if the start day/month or the increase severity day/month is missing.
	Partial	Not a TEAE. The first day of the month and January will be used if the start day/month or the increase severity day/month is missing. The last day of the month and the last month (i.e. December) will be used if the stop day/month is missing.
	Missing	Not a TEAE. The first day of the month and January will be used if the start day/month or the increase severity day/month is missing.
Partial, could be on or after first study drug taken date	Known	Considered as TEAE, if stop date is after first study drug taken date. The first study drug taken date will be used if start/increase severity date is in the same month/year with first study drug taken date, or the first day of the month and January will be used if the start day/month or the increase severity day/month is after first of study drug taken date. Considered as not TEAE, if stop date is prior to first of study drug taken date. The first day of the month and January will be used if the start day/month or the increase

Start/ Increase Severity Date	Stop Date	Action
		severity day/month is missing.
	Partial	Considered as TEAE. The first of study drug taken date will be used if start/ increase severity date is in the same month/year with first study drug taken date, or the first day of the month and January will be used if the start day/month or the increase severity day/month is after first study drug taken date. The last day of the month and the last month (i.e. December) will be used if the stop day/month is missing.
	Missing	Considered as TEAE. The first study drug taken date will be used if start/ increase severity date is in the same month/year with first study drug taken date, or the first day of the month and January will be used if the start day/month or the increase severity day/month is after first study drug taken date.
Missing	Known	Considered as TEAE if stop date is on or after the date of the first dose of study drug.
	Partial	The last day of the month and the last month (i.e. December) will be used if the stop day/month is missing. If the imputed stop date is on or after the first dose of study drug considered as a TEAE; if the year is missing, considered as a TEAE.
	Missing	Considered as a TEAE

APPENDIX 3 CTC Grading for Laboratory Parameters

Laboratory measurements will be graded using the NCI-CTCAE v4.03.

			Grade				
Analytic	**Direction**	**Unit**	**0**	**1**	**2**	**3**	**4**
Hematology							
Hemoglobin	Hypo	g/L	LLN =< Value	100 =< Value < LLN	80 =< Value < 100	Value < 80	
	Hypo	g/dL	LLN =< Value	10 =< Value < LLN	8 =< Value < 10	Value < 8	
	Hypo	mmol/L	LLN =< Value	6.2 =< Value < LLN	4.9 =< Value < 6.2	Value < 4.9	
WBC	Hypo	10^9/L	LLN =< Value	3 =< Value < LLN	2 =< Value < 3	1 =< Value < 2	Value < 1
	Hypo	/mm3	LLN =< Value	3000 =< Value < LLN	2000 =< Value < 3000	1000 =< Value < 2000	Value < 1000
Neutrophils	Hypo	10^9/L	LLN =< Value	1.5 =< Value < LLN	1 =< Value < 1.5	0.5 =< Value < 1	Value < 0.5
	Hypo	/mm3	LLN =< Value	1500 =< Value < LLN	1000 =< Value < 1500	500 =< Value < 1000	Value < 500
Lymphocytes	Hypo	10^9/L	LLN =< Value	0.8 =< Value < LLN	0.5 =< Value < 0.8	0.2 =< Value < 0.5	Value < 0.2
	Hypo	/mm3	LLN =< Value	800 =< Value < LLN	500 =< Value < 800	200 =< Value < 500	Value < 200
	Hyper	10^9/L	Value <= ULC		4 < Value =< 20	20 < Value	
	Hyper	/mm3	Value <= ULN		4000 < Value =< 20,000	20,000 < Value	
Monocytes	NA						
Eosinophils	NA						
Basophils	NA						
Platelet count	Hypo	10^9/L	LLN =< Value	75 =< Value < LLN	50 =< Value < 75	25 =< Value < 50	Value < 25
	Hypo	/mm3	LLN =< Value	75,000 =< Value < LLN	50,000 =< Value < 75,000	25,000 =< Value < 50,000	Value < 25,000
Biochemistry							
Serum creatinine	Hyper	umol/L	Value <= ULN	ULN< Value =< 1.5 × ULN	1.5 × ULN< Value =< 3 × ULN	3 × ULN< Value =< 6 × ULN	6 × ULN< Value
BUN	NA						
Alanine aminotransferase (ALT)	Hyper	U/L	Value <= ULN	ULN < Value =< 3 × ULN	3 × ULN< Value =< 5 × ULN	5 × ULN< Value =< 30 × ULN	20 × ULN< Value
Aspartate aminotransferase (AST)	Hyper	U/L	Value <= ULN	ULN < Value =< 3 × ULN	3 × ULN< Value =< 5 × ULN	5 × ULN< Value =< 30 × ULN	20 × ULN< Value
Alkaline phosphatase (ALP)	Hyper	U/L	Value <= ULN	ULN < Value =< 2.5 × ULN	2.5 × ULN< Value =< 5 × ULN	5 × ULN< Value =< 20 × ULN	20 × ULN< Value
Total bilirubin	Hyper	umol/L	Value <= ULN	ULN < Value =< 1.5 × ULN	1.5 × ULN< Value =< 3 × ULN	3 × ULN< Value =< 10 × ULN	10 × ULN< Value
Albumin	Hypo	g/L	LLN =< Value	30 =< Value < LLN	20 =< Value < 30	Value < 20	

			Grade				
Analytic	**Direction**	**Unit**	**0**	**1**	**2**	**3**	**4**
	Hypo	g/dL	LLN =< Value	3 =< Value < LLN	2 =< Value < 3	Value < 2	
Sodium	Hypo	mmol/L	LLN =< Value	130 =< Value < LLN		120 =< Value < 130	Value < 120
	Hyper	mmol/L	LLN =< Value	ULN < Value =< 150	150 < Value =< 155	155 < Value =< 160	160 < Value
Potassium	Hypo	mmol/L	LLN =< Value	3 =< Value < LLN		2.5 =< Value < 3	Value < 2.5
	Hyper	mmol/L	Value <= ULN	ULN < Value =< 5.5	5.5 < Value =< 6	6 < Value =< 7	7 < Value
Chloride	NA						
	Serology						
HBV	NA						
HCV	NA						
	Urinalysis						
Urine protein	Hyper		Negative	1+	2+		
	Hyper	g/24hrs	Value <= ULN	ULN < Value < 1	1 =< Value =< 3.4	3.5= < Value	
	Pregnancy test						
Pregnancy test	NA						

LLN: lower limit of normal; ULN: upper limit of normal.