A Randomised Open Label Exploratory, Safety and Tolerability Study with PP100-01 in Patients Treated with the 12-hour Regimen of N-Acetylcysteine for Paracetamol/Acetaminophen Overdose

Short Title:
PP100-01 (calmangafodipir) for Overdose of Paracetamol (The POP Trial)

Statistical Analysis Plan

CONFIDENTIAL

Version No	3.0
Date	29th August 2018
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1.0	12th September 2017	New document
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3.0	29th August 2018	Final review prior to database lock as per SOP ECTU_ST_04. Addition of sponsor sign-off and simplification as per sponsor request. Clarification of wordings and correction of typographical errors; reporting of adverse events by system organ class in section 4.4; inclusion of relative change for ALT/INR in section 4.5.

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Contents

List of Abbreviations

Abbreviation	Full name
AE	Adverse Event
ALT	Alanine Aminotransferase
AR	Adverse Reaction
CI	Confidence Interval
CONSORT	CONsolidated Standards Of Reporting Trials
DMC	Data Monitoring Committee
ECG	Electrocardiogram
ECTU	Edinburgh Clinical Trials Unit
FA	Full analysis population
GLDH	Glutamate dehydrogenase
INR	International normalised ratio
IQR	Inter quartile range
Max	Maximum
MCV	Mean cell volume
MedDRA	Medical Dictionary for Regulatory Activities
Min	Minimum
NAC	N-acetylcysteine
NSAID	Non-steroidal anti-inflammatory drug
PP	Per-protocol population
PP100-01	Calmangafodipir
PT	Prothrombin time
Q1	Lower quartile
Q3	Upper quartile
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SAP	Statistical Analysis Plan
SD	Standard Deviation
SOC	System Organ Class
SOP	Standard Operating Procedure
SSRI	Selective serotonin reuptake inhibitor
SUSAR	Suspected Unexpected Serious Adverse Reaction
WBC	White blood cells

1. Introduction

This document details the criteria to be used for the definition of the analysis populations and the statistical methodology for analysis for the POP trial, a randomised open label exploratory, safety and tolerability study of PP100-01 in patients treated with the 12-hour regimen of N-acetylcysteine (NAC) for paracetamol/acetaminophen overdose.

This document has been compiled according to the Edinburgh Clinical Trials Unit (ECTU) standard operating procedure (SOP) "Statistical Analysis Plans" ECTU_ST_04 and has been written based on information contained in the study protocol version 3.0, dated 28 June 2017.

The study will be an open label, randomised, exploratory, rising dose design, phase 1 safety and tolerability study of PP100-01 in patients treated with NAC for paracetamol/acetaminophen overdose. Within each of 3 dosing cohorts of 8 patients, participants will be randomised to PP100-01 plus NAC or NAC alone in the ratio 6:2. The aim is to recruit 24 patients in total.

2. Statistical Methods section from the protocol

12.1 Statistical Analysis Plan
The principal features of the statistical analysis of the data are described in this section. A more technical and detailed elaboration of the principal features will be written in a separate Statistical Analysis Plan (SAP).

12.2 Analysis Data Sets
Full analysis population:
Patients will be included in the full analysis population, the primary population for analysis of efficacy, if they have received any PP100-01 or NAC. Data will be analysed according to the randomised treatment group.

Per protocol population:
The stringent per protocol population includes patients from the full analysis population for whom the study protocol has been followed without any major violations.

Safety population:
The population for safety analysis will be patients who have received any PP100-01 or NAC. Data will be analysed according to the treatment received (NAC plus PP100-01; or NAC alone). Any patient who withdraws during the treatment phase of the study will be included in the safety population (adverse events and laboratory parameters). Data for all patients will be listed, and a list of withdrawn patients, with all reasons for withdrawal, will be given.

Data will also be listed for those patients who, after having consented to participate, underwent baseline examinations required for inclusion into the study but who because a criterion for exclusion was met or for other reasons were not included in the study.

12.3 Estimation of Sample Size
With the clinical safety data that are available we do not expect any adverse events but we note that

PP100-01 has not been administered in paracetamol overdose patients treated with NAC. We deem that 6 patients per group in this initial dose escalation study will allow initial exploration of effects on biomarkers and potential dose limiting toxicity.

12.4 Proposed Statistical Analysis

A CONSORT diagram depicting the flow of participants through the study will be reported. Descriptive statistics will be used to report baseline characteristics by treatment group and overall: continuous variables will be summarised by the mean, standard deviation, median minimum and maximum; categorical variables will be summarised using the number and percentage in each category. Log transformation will be used where appropriate.

We will keep missing data to an absolute minimum, but where there is missing data those records will be removed from the analysis; if missing data rates are substantial the effect of this will be investigated using sensitivity analyses.

Binary outcomes (including the primary outcome) will be reported by treatment group and overall using the proportion and exact 95% confidence interval. Binary outcomes will be compared within dosing cohort between the PP100-01+NAC and the NAC alone patients using a difference in proportions and its exact 95% confidence interval. Binary outcomes will be compared between each of PP100-01 dosing groups A, B and C and the combined NAC alone group in the same way.

Continuous outcomes will be reported by treatment group and overall using the mean and 95% confidence interval. Continuous outcomes will be compared within dosing cohort between the PP100-01+NAC and the NAC alone patients using the difference in means and its 95% confidence interval. Continuous outcomes will be compared between each of PP100-01 dose groups A, B and C and the combined NAC alone group in the same way. The continuous outcome analyses listed above will be repeated using the change from baseline in each continuous outcome.

3. Overall Statistical Principles

3.1 SAP objectives

The objective of this SAP is to describe the statistical analyses contributing to the final report and publication(s) of the POP trial. Analyses of the experimental biomarkers such as CK18, microRNA MiR122, GLDH, mitochondrial DNA are not included and will be covered in a separate analysis plan.

3.2 General principles

In general terms, categorical data will be presented using counts and percentages, whilst continuous variables will be presented using the mean, standard deviation (SD), median, minimum, maximum and number of patients with an observation (n), using a format similar to example tables 1 and 2 below. Data will be summarised overall and split by PP100-01 treatment group (NAC alone; NAC + 2μmol/kg PP100-01; NAC + 5μmol/kg PP100-01; NAC + 10μmol/kg PP100-01) and timepoint, where applicable.

Example 1. Data presentation for categorical data

Variable	Timepoint	Category	NAC alone	NAC + 2µmol/kg PP100-01	NAC + 5µmol/kg PP100-01	NAC + 10µmol/kg PP100-01	Overall
			N=xx	N=xx	N=xx	N=xx	N=xx
Variable A	Timepoint x	Category 1	xx (%)	xx (%)	xx (%)	xx (%)	xx (%)
		...	...	...	...	...	...
		Category n	xx (%)	xx (%)	xx (%)	xx (%)	xx (%)

Example 2. Data presentation for continuous data

Variable	Timepoint / visit	Statistic	NAC alone	NAC + 2µmol/kg PP100-01	NAC + 5µmol/kg PP100-01	NAC + 10µmol/kg PP100-01	Overall
			N=xx	N=xx	N=xx	N=xx	N=xx
Variable A	Timepoint x	Mean (SD)	Xx	xx	xx	Xx	xx
		Median	Xx	xx	xx	Xx	xx
		Min, Max	xx, xx	xx, xx	xx, xx	xx, xx	xx, xx
		n	Xx	xx	xx	Xx	xx

Two-sided 95% confidence intervals (CIs) will be presented.

Where there are missing data for an outcome variable, in the first instance, a complete case analysis will be performed for that outcome, unless otherwise specified. In tabulations, numbers of missing observations will be provided, but percentages will not include them.

Normality will be examined by normal probability plots. If the distributional assumptions for the parametric approach are not satisfied, further data transformation (to alleviate substantial skewness or to stabilise the variance), or other suitable methods will be considered. This will be documented in the statistical results report together with the reasoning supporting the action taken, if applicable.

All analyses and data manipulations will be carried out using SAS [1].

3.3 Analysis Populations

The analysis populations defined for the statistical reporting of the trial are as follows:

Full analysis population (FA) Patients will be included in the full analysis population, the primary population for analysis of efficacy, if they have received any PP100-

01 or NAC. Data will be analysed according to the randomised treatment group.

Per protocol population (PP) The stringent per protocol population includes patients from the full analysis population for whom the study protocol has been followed without any major violations.

Safety population The population for safety analysis will be patients who have received any PP100-01 or NAC. Data will be analysed according to the treatment received (NAC plus PP100-01; or NAC alone). Any patient who withdraws during the treatment phase of the study will be included in the safety population.

4. List of Analyses

4.1 Recruitment and retention

The date of first and last patient randomised, and the numbers of patients identified, eligible and randomised, will be reported.

A CONSORT flow diagram will be provided by the POP Trial Manager.

This will present the number (%) of patients, split by treatment group, as follows:

- Randomised
- Receiving at least one dose of study medication
- Completing the trial
- Discontinuing from the trial

In addition, reasons for non-inclusion in the study (prior to randomisation) will be categorised.

The number of patients discontinued early from the study will be summarised by reason for withdrawal and by treatment group.

4.2 Baseline characteristics (FA)

The baseline patient characteristics listed in this section (recorded at either Screening, Timepoint 0 or Baseline [Pre PP100-01]) will be summarised by randomised treatment and overall for the FA population.

- Participant details
  - Age (years)
  - Height (cm)
  - Weight (kg)
  - Body Mass Index (kg/m^2)
  - Ethnicity
  - Gender (male/female)

- Childbearing potential (yes/no) [Female participants only]

- Overdose details
  - Time from paracetamol ingestion to presentation at hospital (hrs)
  - Type of overdose (acute within 8hrs of NAC starting / acute more than 8hrs before NAC starting / staggered intentional / supratherapeutic / not known)
  - Amount of paracetamol ingested known (yes/no)
  - Total paracetamol ingested (g)
  - Other drugs ingested
    - Anticoagulants
    - Non-opioid analgesics
    - NSAIDs
    - Cardiovascular drugs
    - Alcohol
    - Opioids
    - SSRIs
    - Tricyclic antidepressants
    - Benzodiazepines
    - Other

- Concomitant medications during preceding 30 days
  (tabulated by category and whether ongoing)

- Physical examination
  (each classified as Normal / Abnormal not Clinically Significant / Abnormal Clinically Significant)
  - Cardiovascular
  - Respiratory
  - Gastrointestinal

- Vital signs
  - Systolic blood pressure (mmHg)
  - Diastolic blood pressure (mmHg)
  - Pulse (bpm)
  - Respiratory rate (per minute)
  - Temperature (°C)
  - Oxygen saturation (%)

- ECG (Normal / Abnormal not Clinically Significant / Abnormal Clinically Significant)

- Clinical bloods
  - Paracetamol level (mg/L)
  - INR
  - PT (sec)
  - ALT (U/L)
  - Alkaline phosphatase (U/L)
  - Bilirubin (µmol/L)
  - Creatinine (µmol/L)
  - Haemoglobin (g/L)
  - Urea (mmol/L)
  - MCV (fL)

- WBC ($x10^9$/L)
- Sodium (mmol/L)
- Potassium (mmol/L)

No formal statistical testing of baseline characteristics will be performed.

4.3 Adherence with allocated treatment (FA)

No formal statistical testing will be performed. The following will be summarised by treatment group and overall:

- Numbers of patients who were randomised but never treated with either NAC or PP100-01.

- NAC treatment
  - Time from ingestion of paracetamol to start of NAC treatment (hrs)
  - 12 hour NAC regimen
    - Completed without stopping
    - Completed with stop(s) and restart(s)
    - Stopped permanently during first infusion
    - Stopped permanently during second infusion
    - Not started
  - Percentage of intended dose received
    (see Appendix 1 for weight-based lookup table for intended dose)
  - Number of additional NAC infusions after 12 hour regimen
    (none / 1 / 2 / more than 2)

- PP100-01 treatment
  - Time from ingestion of paracetamol to start of PP100-01 treatment (hrs)
  - Bolus status
    - Completed
    - Stopped early
    - Not started
  - Percentage of intended dose received
  - PP100-01 level at 2, 10 and 20hrs

4.4 Primary Outcome (Safety population)

The number and percentage of patients experiencing an adverse event (AE) will be summarised by treatment group and overall. Further tabulations in the same format will be reported for subsets of AEs according to the following criteria:

- Serious adverse events (SAEs)
- AEs starting after the commencement of NAC treatment and within 7 days of consent
- SAEs starting after the commencement of NAC treatment and within 7 days of consent
- Intensity (mild/moderate/severe)
- Treatment given in response to AE (yes/no)

- Outcome (recovered/improved/unchanged/deterioration/death)
- Relationship to NAC (unrelated/possibly related/probably related/definitely related)
- Relationship to PP100-01 (unrelated/possibly related/probably related/definitely related)
- Action taken with NAC (none/interrupted/stopped entirely)
- Action taken with PP100-01 (none/interrupted/stopped entirely)[1]
- Unexpected with, and related to, NAC
- Unexpected with, and related to, PP100-01
- Suspected unexpected serious adverse reaction (SUSAR), subcategorised into SUSAR to NAC; SUSAR to PP100-01; SUSAR to NAC and PP100-01

The number and percentage of patients experiencing an adverse event will also be reported according to MedDRA system organ class (SOC). Results will be presented separately for AEs not classified as serious, for SAEs and for all AEs. Events will be summarised by (a) treatment group and overall, and (b) by treatment received (NAC or PP100-01+NAC) within each dosing cohort.

Primary analysis
The numbers of participants experiencing AEs and SAEs will be reported by treatment group and overall using the proportion and exact 95% confidence interval. AE and SAE proportions will be compared within each dosing cohort between the PP100-01+NAC and the NAC alone patients using a difference in proportions and its exact 95% confidence interval. AE and SAE rates will be compared between each of the PP100-01 2μmol/kg, 5μmol/kg and 10μmol/kg groups and the combined NAC alone group in the same way.

If the limited sample size or a small number of observed events leads to a lack of interpretability of the formal analysis confidence intervals, the analysis of the primary outcome will be restricted to descriptive summaries only.

Missing data
We do not anticipate missing data impacting on the analysis of the routinely recorded adverse event data that form the primary outcome.

4.5 Efficacy Secondary Outcomes (FA,PP)

Hepatotoxicity will be assessed using ALT and INR.

Descriptive summaries will be provided by treatment group and overall at each measurement time point during the treatment period (10 hours, 20 hours). Change from baseline to each of the 10 and 20 hour time points will be summarised by treatment group and overall for ALT and INR.

A graphical summary of each marker will be presented in a separate panel for each treatment group. Each will show the profile of the measurements for individual patients overlaid by the treatment group mean profile.

At each measurement time point during the treatment period (10 hours, 20 hours) ALT and INR will be analysed by treatment group and overall using the mean and 95% confidence interval. They will be compared within each dosing cohort between the PP100-01+NAC and the NAC alone patients using

[1] Note that PP100-01 is administered as a bolus and therefore cannot be stopped once the bolus has been given

the difference in means and its 95% confidence interval. The markers will be compared between each of the PP100-01 2μmol/kg, 5μmol/kg and 10μmol/kg groups and the combined NAC alone group in the same way. These analyses will be repeated using the change from baseline in ALT and INR, for absolute and relative change as appropriate.

Use of the natural log transformation of the data and the geometric mean summary statistic will be considered as appropriate in the descriptive, graphical and formal analyses of these markers.

Paracetamol level (mg/L) will be summarised descriptively and graphically by treatment group and overall at its baseline and 10 and 20hrs measurement time points in the same way as for the ALT and INR markers.

The following efficacy indicators will be summarised by treatment group and overall in the same manner as for the primary outcome analysis in Section 4.4.

- Proportion of patients with paracetamol/acetaminophen concentration >20 mg/L at 10 hrs
- Proportion of patients with paracetamol/acetaminophen concentration >20 mg/L at 20 hrs
- Proportion of patients with a 50% increase in ALT after 10 hrs, compared with baseline value
- Proportion of patients with a 50% increase in ALT after 20 hrs, compared with baseline value
- Proportion of patients who have doubled their ALT after 10 hrs, compared with baseline value
- Proportion of patients who have doubled their ALT after 20 hrs, compared with baseline value
- Proportion of patients with ALT>100 U/L at 10hrs
- Proportion of patients with ALT>100 U/L at 20hrs
- Proportion of patients with INR>1.3 at 20 hrs
- Proportion of patients with INR>1.3 at 20 hrs
- Proportion of patients with ALT>1000 U/L at any time

4.6 Safety Secondary Outcomes (Safety population)

Vital signs (systolic blood pressure, diastolic blood pressure, pulse, respiratory rate, temperature, oxygen saturation) and haematology and clinical biochemistry parameters (PT, alkaline phosphatase, bilirubin, creatinine, haemoglobin, urea, MCV, WBC, sodium, potassium) will be summarised and analysed at their measurement time points using methods corresponding to those described in Section 4.5.

The ECG result (Normal / Abnormal Not Clinically Significant / Abnormal Clinically Significant) will be summarised by treatment group and overall at 2.5, 10 and 20 hours. The ECG summary results at each of these time points will also be cross-tabulated against the baseline ECG result, by treatment group and overall.

The number and proportion of participants who have anaphylactoid reactions recorded will be summarised by treatment group and overall in the same manner as for the primary outcome analysis in Section 4.4.

4.7 Concomitant Medication (FA)

The number and percentage of patients will be presented by treatment group and category of concomitant medication. Concomitant medications will be summarised overall, as well as separately for those (i) recorded in the 30 days preceding randomisation; (ii) recorded as ongoing at baseline and (iii) those started on or after the date of consent.

4.8 Disposition and follow-up (FA,PP)

Duration of hospital stay (hours) will be reported by treatment group and overall using the mean and 95% confidence interval. Duration of stay will be compared between each of the PP100-01 2μmol/kg, 5μmol/kg and 10μmol/kg groups and the combined NAC alone group using the difference in means and its 95% confidence interval.

The cumulative incidence of the following events at 7, 30 and 90 days after randomisation will be summarised by treatment group and overall:

- representation to hospital for any reason
- representation to hospital with liver injury
- repeat overdose
- transfer to liver transplantation unit
- death

4.9 Data listings

Consented patients
Data will be listed for those patients who, after having consented to participate, underwent baseline examinations required for inclusion into the study but who, because a criterion for exclusion was met or for other reasons, were not included in the study. Patient ID and reason(s) for exclusion will be listed.

Withdrawals
Patient ID and all reasons for withdrawal will be listed for patients who have withdrawn from the study.

Medical History (FA)
Medical history will be presented in a line listing, ordered by treatment group and patient ID, of the descriptive text of each event/condition and whether it was classified as past medical history or a presenting complaint.

Adherence with allocated treatment (FA)
Data listings will be provided, ordered by treatment group and patient ID, of the reasons for:

- Temporary stopping of NAC treatment
- Permanent stopping of NAC treatment
- PP100-01 not being given or PP100-01 being stopped early

Protocol deviations and violations (FA)
A listing of protocol deviations and violations will be provided, ordered by treatment group, patient ID and study time point.

Primary outcome (Safety)
Details of adverse events will be listed, ordered by treatment group, patient ID and start date. The listing will include:

- Event category (as recorded on CRF)
- Start date and time
- Date and time resolved
- Seriousness (yes/no)
- SUSAR (yes/no)
- Intensity (mild/moderate/severe)
- Treatment given in response to AE (yes/no)
- Outcome (recovered/improved/unchanged/deterioration/death)
- Relationship to NAC (unrelated/possibly related/probably related/definitely related)
- Relationship to PP100-01 (unrelated/possibly related/probably related/definitely related)
- Action taken with NAC (none/interrupted/stopped entirely)
- Action taken with PP100-01 (none/interrupted/stopped entirely)
- Expectedness with NAC (expected/unexpected/NA)
- Expectedness with PP100-01 (expected/unexpected/NA)

Concomitant Medication (FA)
Three concomitant medication data listings will be provided, one for medications in the 30 days preceding randomisation; the second for medications recorded as "ongoing" at baseline; and a third for those started on or after the date of consent. Each listing will be ordered by treatment group, patient ID and start date, and will contain: Patient ID; Randomisation date; Category; Medication; Start date; Stop date; Ongoing; Dose & Units; Frequency; Route.

5. Derived variables

5.1 Body Mass Index (BMI)

Body mass index will be calculated based on weight(kg) and height (m):

$$BMI=weight/(height)^2$$

6. Validation and QC

The following will be performed by a second ECTU statistician:

1. Separate programming and checking of primary outcome results and conclusions.
2. The statistical report will be read and sense-checked.

7. Data sharing

A set of files containing the final trial analysis data sets will be prepared in .CSV format, along with a data dictionary. The files will be made available to the Chief Investigator following the preparation of the final statistical report.

8. References

1. SAS® Institute Inc. SAS for Windows. SAS Institute Inc.: Cary, NC, U.S.A

9. Appendix

Appendix Table 1 Doses of NAC in 12 hour regimen

Patient weight (kg)	First infusion – Volume of NAC (mL)	Second infusion – Volume of NAC (mL)
30 - 39	18	35
40 - 49	23	45
50 - 59	28	55
60 - 69	33	65
70 - 79	38	75
80 - 89	43	85
90 - 99	48	95
100 - 109	53	105
≥110	55	110