UCL

| | |
|---|---|
| Full title of trial | A pilot, open label, phase II clinical trial of nebulised recombinant tissue-Plasminogen Activator (rtPA) in patients with COVID-19 ARDS: The Plasminogen Activator COVID-19 ARDS (PACA) trial |
| Short title | Nebulised rtPA for ARDS due to COVID-19 – The PACA trial |
| Sponsor | University College London (UCL) |
| Sponsor protocol number | 132151 |
| EudraCT No | 2020-001640-26 |
| ISRCTN / Clinicaltrials.gov no | NCT04356833 |
| **Document name** | **PACA**<br>**Statistical Analysis Plan for Cohort 2** |
| **Document date** | **25 Feb 2021** |

# Nebulised rt-PA for ARDS due to COVID-19 – The PACA trial

# Statistical Analysis Plan for Cohort 2

**Version History Log**

| Version | Date | Changes |
|---|---|---|
| 1.0 | 25 Feb 2021 | Final version 1.0 |

**SAP Authorisation**

| Author | |
|---|---|
| Name: | Marie Watissée (WStats Limited) |
| Role: | Study Statistician (cohort 2) |
| Signature: | DocuSigned by: 03777597953E4CD... |
| Date: | 25/02/2021 |

| Approver | |
|---|---|
| Name: | Dr Pratima Chowdary |
| Role: | Chief investigator |
| Signature: | DocuSigned by: Pratima Chowdary 624D7161F07E422... |
| Date: | 2/25/2021 |

**Contents**

**List of Abbreviations**

| | |
|---|---|
| CPAP | Continuous positive airway pressure |
| CRF | Case report form |
| DMP | Data Management Plan |
| IMV | Invasive mechanical ventilation |
| NIV | Non-invasive ventilation |
| PCR | Polymerase chain reaction |
| rt-PA | Recombinant tissue plasminogen activator |
| SAP | Statistical analysis plan |
| SOFA | Sequential organ failure assessment |
| WHO | World Health Organization |

# 1. Introduction

## 1.1 Purpose and scope of the statistical analysis plan

This document outlines the statistical methods to be implemented for the final analyses of the data from cohort 2 of the PACA trial. The purpose of this plan is to provide specific guidelines from which the analyses will proceed. Any deviations from these guidelines will be documented in the statistical report.

This document is based on:

- The study protocol, version 10.0, dated 2 February 2021,
- A separate Statistical Analysis Plan (SAP) developed for the analysis of cohort 1 of the PACA trial, version 1.0 dated 18 November 2020.
- The Case Report Form (CRF) version 1.0, dated 29 January 2021
- The Data Management Plan (DMP), version 3.0 dated 3 February 2021.

## 1.2 Analysis organisation

**Final analysis**

The final analysis will be initiated after the last participant has completed follow-up, all relevant data has been entered, checked, and locked, the analysis plan has been finalised and approved, and the analysis programs prepared as much as possible.

**A statistical report will be produced. The report will contain in-text summaries of data and graphical outputs prepared in accordance with this statistical analysis plan. No separate tables or data listings will be produced.**

**Preliminary Interim results**

Given the expected decrease in cases during the second wave of COVID-19 in February 2021 in the UK, it may become difficult to continue recruitment. A datacut will therefore be performed after 20 patients have completed follow-up, after all their relevant data has been entered, and checked as much as possible.

A limited subset of summaries and graphical outputs will be prepared in accordance with this statistical analysis plan and may be shared with the study Data Monitoring Committee, Trial Management Group and UK Research and Innovation, Government Department.

This datacut was not planned in the protocol, and these results will be considered interim and preliminary and their distribution will be limited by the Chief Investigator as much as possible. Given the open-label design and the descriptive nature of the study, this interim look at the data is not considered to risk the integrity of the study, however, results will have to be interpreted with caution and no conclusions will be drawn until the final data is cleaned, locked, and final analyses performed.

## 1.3 Data checking

Before analysis, basic checks will be performed to check the quality of the data. Incomplete or inconsistent data include:

- Missing data;

- Data outside expected range;
- Other inconsistencies between variables, e.g., in the dates the questionnaires were completed.

If any inconsistencies are found, the corresponding values will be double checked with the researchers, study team and corrected in the database if necessary. Checks are detailed in the DMP for the study.

Any further changes made during analyses will be documented by the trial statistician.

## 2 Trial Objectives (from Protocol)

### 2.1 Primary objectives

1. Efficacy: Investigate the potential for efficacy of nebulised rt-PA in patients presenting with severe COVID-19 requiring Invasive mechanical ventilation (IMV) or non-invasive support with NIV OR continuous positive airway pressure (CPAP) OR high flow oxygen OR standard oxygen therapy;
2. Safety: Evaluate the safety of nebulised rt-PA treatment.

### 2.2 Secondary objectives

1. Investigate the impact on patient's clinical status over time using the WHO ordinal scale of clinical improvement;
2. Investigate the effect of nebulised rt-PA on other respiratory markers (such as lung compliance) and organ dysfunction;
3. Investigate the impact on in hospital mortality and resource utilisation.

### 2.3 Exploratory objective

Changes to coagulation and inflammatory markers concerning intervention and response to treatment.

## 3 Trial Design

This study is a phase II, open label, multicentre, uncontrolled, repeated dose, pilot trial of nebulised rt-PA in patients with COVID-19 ARDS. The complete trial design for both cohorts is detailed in the study protocol.

Cohort 2 of the study will aim to recruit more patients during the second surge of COVID-19 underway in early 2021, to provide more data on the safety of rt-PA. Based on the analysis of cohort 1, fewer timepoints will be collected which will allow for more rapid recruitment while at the same time not compromising safety monitoring. A more flexible dosing regimen for rt-PA will be utilised which is described in section 7.3.2 of the protocol.

The study will recruit patients requiring either IMV or non-invasive oxygen support. Eligible patients (or if patients lack capacity, their legal representative) will be provided with an information sheet and informed consent will be sought. Eligibility will be assessed via routine clinical assessments, which may have been done prior to consent. The only exceptions are a pregnancy test (blood or urine), and possibly any assessments that were not done as per routine

care. These must be done following consent, and all screening assessments must have been done during the 24-hour period before dosing with rt-PA. Patients will be followed up until discharge, death, or day 28 whichever is sooner.

### 3.1 Sample size calculation

The initial sample size for this pilot study was not based on a statistical consideration. The recruitment target of 12 patients treated with rt-PA and Standard of care was based on feasibility and the planned recruitment rate.

During the first surge 9 patients were recruited to the pilot study, cohort 1, with 6 patients receiving IMV and 3 patients receiving non-invasive oxygen support. When the first surge died out, recruitment was not feasible and following discussion with DMC and TMG cohort 1 was closed for further recruitment and historical controls were recruited for evaluation of efficacy to enable a sample size calculation for a larger phase 3 study.

For cohort 2, the sample size of 30 patients is similarly not based on statistical consideration but has been selected on the grounds of what is reasonable amid a second surge of COVID-19 admissions in early 2021 and the number of patients we might reasonably accept to recruit across the 2 sites. 30 patients will be recruited in total, with an aim to recruit a minimum of 10 IMV patients and 10 patients on non-invasive oxygen support.

### 3.2 Randomization

Not applicable

## 4 Data collection

The complete information on data collection for both cohorts is detailed in the study protocol. The sections below only refer to cohort 2 of the study.

Data will be collected on study-specific case report forms (CRFs). Source data are contained in source documents (medical notes and laboratory reports) and must be accurately transcribed on to the CRF.

Demographic information, medical history, concomitant medication and other baseline information will be collected.

### 4.1 Primary outcome measures

Efficacy

Arterial oxygen saturation levels, specifically arterial hypoxemia using the arterial oxygen to inhaled oxygen ratio (PaO2/FiO2 or SaO2/FiO2) measured at multiple timepoints over the study period: baseline, during treatment, end of treatment, 3 days post end of treatment and 5 days post end of treatment.

PaO2/FiO2 will be a calculated field on the trial database derived from the fields for PaO2 and FiO2. The PaO2/FiO2 ratio for NIV patients is calculated by converting SpO2 into PaO2 using the conversion tables found in the protocol appendix. FiO2 is calculated by converting the oxygen flow rate (in L/min) into FiO2, using the table that is appropriate for the ventilation type. The

values required for the conversation (SpO2 and oxygen flow rate) are recorded on worksheets and CRFs. For cohort 2, the imputed PaO2 and FiO2 will be entered on the CRF and also the database.

For cohort 2, respiratory status of patients is monitored hourly and data will be extracted once a day coinciding with the worst PF ratio. As PF ratio is the primary endpoint, in addition to the worst PF ratio, up to a maximum of 5 data points spread across 24 hrs that represents patients' clinical status will be extracted (6 in total).

Safety

Review of Adverse events and Adverse drug reactions including bleeding episodes will be done daily until 5 days after the last dose, and then weekly (+/- 1 day) until day 28, discharge or death whichever occurs first.

Plasma Fibrinogen levels will be measured locally at a minimum every other day for cohort 2, until 5 days after the last dose, and then as required until day 28, discharge or death whichever occurs first.

### 4.2 Secondary outcome measures

Lung compliance (defined as tidal volume / (peak inspiratory pressure – PEEP)) as part of respiratory status is monitored hourly and data will be extracted once a day coinciding with the worst PF ratio.

Clinical status as assessed by a 7-point WHO ordinal scale will be recorded at baseline and daily up to 5 days post end of treatment and at day 28, discharge or death (whichever comes first)

Daily Sequential Organ Failure Assessment (SOFA) score will be recorded at baseline through up to 5 days post end of treatment.

In hospital mortality will be recorded.

### 4.3 Exploratory measures

Levels of plasma biomarkers of pro- and anti-inflammatory markers (interleukin-6, interleukin-8, interleukin-10 and interleukin-1Ra), endothelial injury (plasma von Willebrand factor) coagulation (procoagulants, anticoagulants, and fibrinolytic pathway) measure of tissue damage will be measured as per the protocol schedule of assessments.

## 5 Data Analysis Plan

### 5.1 General statistical considerations

All analyses will be descriptive in nature and should be considered as hypothesis generating rather than providing firm conclusions.

Analyses for both primary and secondary outcomes will be conducted following an Intention To Treat approach, and non-compliance will be reported at the individual patient level.

No adjustments for missing data will be made. Partial or incomplete dates are imputed at the database level, as per the study DMP.

All relevant data will be tabulated and summarised separately for the groups of Cohort 2:

- Patients receiving IMV at enrolment (Group 5, patient numbered 501 onwards)
- Other patients with mild ARDS requiring non-invasive oxygen support or standard oxygen therapy at enrolment (Group 6, patient numbered 601 onwards)

Data will also be tabulated overall where appropriate. Data will be presented using standard descriptive statistics. Continuous variables will be summarised using number of observations, mean, and standard deviation, median, inter-quartile range, minimum, and maximum values. Categorical values will be summarised using number of observations and percentages.

## 5.2 Data Derivations and definitions

| | |
|---|---|
| Baseline | For patients treated with rt-PA + Standard of care, the last available value prior to the first administration of rt-PA will be considered the baseline value. |
| Treatment day 1 | Day of the first administration of rt-PA |
| Treatment day | Calculated for assessment post baseline as: (date of assessment – date of Treatment Day 1) +1<br>Calculated for assessment performed pre-treatment as: (date of Treatment Day 1 - date of assessment) |
| PaO2/FiO2 ratio | computed in the database as described in Section 4.1 and in the study DMP |
| Worst daily PaO2/FiO2 ratio | The lowest PaO2/FiO2 ratio recorded for a patient on a specific day |
| The first 28 days | Defined as Treatment Day 1 to Treatment Day 28 |
| Oxygenation free day | A patient's study day will be identified as an oxygen-free day, if the oxygen support for that day was reported as 'Room Air' at all times collected in the Ward. |
| Ventilator-free day | A patient's study day will be identified as an ventilator -free day, if the oxygen support for that day was not reported as 'IMV' at any time that day |
| New oxygen use | A patient/day record will be identified as a new oxygen use, if the oxygen support for all days recorded prior to that point was reported as 'Room Air' |
| New mechanical ventilation use | A patient/day record will be identified as a new mechanical ventilation, if this the first occurrence of oxygen support reported as 'MV' at any time that day. |
| Lung compliance | computed in the database using tidal volume / (peak inspiratory pressure – PEEP) in the study DMP |

## 5.3 Summary of baseline characteristics

All baseline values of the outcomes will be summarised using descriptive statistics.

Data to support a **CONSORT flow chart** will be provided (Table 1). This will include the number of eligible participants, number of participants agreeing to enter the trial, the number of patients in each group that were enrolled, assessed for eligibility, excluded (with reasons listed), assigned to a group, treated with rt-PA , followed up, discontinued (with reasons such as lost to follow-up,

withdrawn, or death), and number of patients analysed in each group, and where applicable excluded from analyses (with reasons).

**Demographic characteristics** will be summarised (Table 2):

- Age at consent,
- Gender,
- Ethnicity (White Caucasian, Black or African American, Asian, Native Hawaiian/ Pacific Islander, American Indian/ Alaskan Native, Hispanic or Latino, Not Available/ Not Reported, Other)
- Height in cm,
- Weight in kg,
- BMI in kg/m2,

**Other Admission data** will be summarised (Table 3):

- Number of days since symptoms onset,
- Number of days since hospital admission,
- Number of days since ICU admission,
- Chest X-ray results (abnormal- suggestive of COVID-19, abnormal- likely COVID-19 , abnormal- other, normal)
- CT Pulmonary Angiogram results (Evidence of COVID 19 Pneumonia, Evidence of pulmonary thrombi, Evidence of RV strain)

**Physical examination at admission**, by body system, with frequencies and percentages and **Medical history** will also be summarised (Tables 4 and 5).

### 5.4 Follow-up and Exposure

The study duration and treatment durations (in days) will be calculated for each subject and summarised (Table 6).

The numbers of missed doses and number of doses interruptions will also be summarised.

### 5.5 Requirement for oxygenation and ventilation support

The ventilation type received at enrolment will be summarised. Any change in requirement for oxygenation and ventilation support will be summarised (Table 7): the number of patients switching from IMV to NIV, and the number of patients moved to IMV from NIV at enrolment will be summarised.

The length of ventilation (days) of each ventilation type will be summarised.

A graphical display will be used to show all the recorded changes in ventilation support (Figure 1).

Patients receiving CPAP or High Flow Nasal O2 will be looked at in particular and additional summaries may be produced.

### 5.6 Analysis of primary outcome

Efficacy

The primary outcome for efficacy is oxygenation, measured using the **PaO2/FiO2 ratio** over time.

Spaghetti plots will be used to show all the recorded PaO2/FiO2 for patients over time (Figure 2).

The worst daily PaO2/FiO2 ratio measured over time will be described for each group, and overall (Table 8, Figure 3). The change from baseline in the worst daily PaO2/FiO2 ratio will be summarised and plotted at each post baseline timepoint (Table 9, Figure 4).

If necessary, appropriate transformations of the data will be performed (e.g., using the natural logarithm).

Changes in worst daily PaO2/FiO2 ratio over time will need to be interpreted taking into consideration that a patient's condition may have changed and led to their ventilation type changed post enrolment. If data allows, additional descriptions of the PaO2/FiO2 ratio over time may be performed in each subgroup of patients experiencing such changes (e.g. IMV to NIV, or vice versa).

Safety

For treatment safety, the incidence and severity (either major or minor) of **bleeding events** will be summarised with frequencies and percentages in each group (Table 10).

In addition, the incidence by cause of bleed (traumatic or spontaneous), and the incidence of treated bleeds will be summarised (Table 10).

Spaghetti plots will be used to show all the **fibrinogen levels** for patients over time (Figure 5). Mean change from baseline in daily Fibrinogen levels will be summarised and plotted at each post baseline timepoint (Table 11, Figure 6).

Patients receiving tocilizumab will be looked at, and additional summaries may be produced in this subset.

The incidence of **serious adverse events** will be summarised (Table 12).

### 5.7 Analysis of secondary outcomes

All secondary outcomes measured overtime will be summarised at screening and each post-baseline timepoint, for each group, and overall where appropriate:

- Clinical status as assessed by the 7-point ordinal scale (Table 13)
- Change in clinical status will be described by the number of days spent in each WHO ordinal scale class (Table 13, Figure 7)
- Changes in lung compliance from baseline and absolute values at day 5 (96 hrs ± 2 hrs), day 7 (144 hrs ± 4hrs), end of treatment, 3 and 5 days post end of treatment (Table 14).
- SOFA score and change from baseline (Table 15)

The following secondary outcomes will also be summarised for each group, and overall, where appropriate (Table 16):

- Number of oxygenation free days in the first 28 days
- Number of ventilator-free days in the first 28 days

- The incidence and number of days of new oxygen use, non-invasive ventilation, or high flow oxygen devices in the first 28 days.
- Incidence and number of days of new mechanical ventilation use in the first 28 days
- Intensive care unit free days in the first 28 days
- Length of ICU stay post treatment

All these summaries will be presented as absolute number of days as well as percentage considering the length of follow-up for each patient, for example, the proportion of oxygen-free days during follow-up will be calculated.

The number of patients with use of vasopressors at any time during the study will be summarised (Table 17)

### 5.8 Analysis of other outcomes

Respiration rate has been identified as an important measure and will be summarised and plotted in line with the primary outcome (Table 18, Figure 10, 11).

Spaghetti plots may be used to show other data such as Glasgow scale, Vital signs, Respiratory data, laboratory data and coagulation results (Figure 12.x).

Concomitant medications will be codified into consistent terminology in text format as part of the data cleaning prior to analysis and may be summarised.

## 6 Software

The statisticians will receive a copy of the data from the trial database, exported to comma separated values files by the data manager for the study. All the statistical analysis will be performed using SAS version 9 (or above) and/or R version 4.0.2 (or above).

## 7 References

None

**Trial Statistician's Engagement letter**

Marie Watissée
WStats Limited
mwatissee@WStats.co.uk

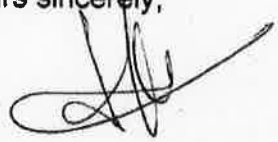
*Dated 8 Jan 2021*

Dear UCL JRO Sponsor Regulatory Manager

**Re:** A pilot, open label, phase II clinical trial of nebulised recombinant tissue-Plasminogen Activator (rtPA) in patients with COVID-19 ARDS: The Plasminogen Activator COVID-19 ARDS (PACA) trial
**UCL Chief Investigator:** Professor Pratima Chowdary

This letter is to confirm that, subject to contract, I will be the trial statistician for the above study and will provide oversight for all statistical aspects for Cohort 2 of this trial. I have reviewed the statistical parts of the most recent protocol (version 8.0 including comments dated 7Jan2021) and I am satisfied that I can provide the statistical support required for this trial. I plan to continue my involvement through to completion and publication stages.

Yours sincerely,



Marie Watissée