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

Statistical Analysis Plan

Version History Log

Version	Date	Changes
0.1	28/04/2020	
0.2	29/04/2020	Minor edits
0.3	01/05/2020	Added details on the nature & analyses of outcomes
0.4	26/10/2020	Modified to account for historical controls
0.5	05/11/2020	Minor edits
0.6	16/11/2020	Added NEWS 2 score
1.0	18/11/2020	Minor edits

Contents

1. Study Summary

For full details see the protocol (version 7.0, 30/10/2020).

Title	A pilot, open label, phase II clinical trial of nebulised recombinant tissue-Plasminogen Activator (rt-PA) in patients with COVID-19 ARDS: The Plasminogen Activator COVID- 19 ARDS (PACA) trial.
Short title	Nebulised rt-PA for ARDS due to COVID-19 – The PACA trial
Chief Investigator	Pratima Chowdary
Statisticians	Aidan O'Keeffe & Federico Ricciardi
Health economist	…
Design	Phase II, open label, multi-centre, pilot interventional study.
Primary objectives	1. Efficacy: Investigate the potential for efficacy of nebulised rtPA in patients presenting with severe COVID-19 requiring 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 rtPA treatment.
Primary Outcomes	Efficacy 1. Change in PaO_2/FiO_2 ratio from baseline (defined as 3 days prior to treatment), daily during treatment, end of treatment and 3 and 5 days post treatment in the groups receiving rtPA Safety 1. Incidence and severity of major bleeding events directly attributable to the study drug 2. Decrease in fibrinogen levels to < 1.5 gm/L during treatment period and 48 hrs after the last dose of treatment 3. Number and nature of serious adverse events causally related to the treatment
Population	<u>Inclusion criteria</u> 1. Patients with COVID-19 confirmed by PCR 2. ≥16 years 3. Willing and able to provide written informed consent or where patient doesn't have capacity, consent obtained from a legal representative 4. Patients on IMV must meet both the following criteria: a. PaO2/FiO2 of ≤ 300 b. Intubated > 6 hrs 5. Patients on NIV must meet all the following criteria: a. PaO2/FiO2 ≤ 300 or equivalent imputed by non-linear calculation from SpO2/FiO2 b. In-patient > 6 hours and being actively treated

	c. On support with non-invasive ventilation OR continuous positive airway pressure (CPAP) OR high flow OR standard oxygen therapy. Exclusion criteria 1. Females who are pregnant 2. Concurrent involvement in another experimental investigational a. medicinal product 3. Known allergies to the IMP or excipients of IMP 4. A pre-existing bleeding disorder (e.g. severe haemophilia) 5. Pre-existing severe cardiopulmonary disease (e.g. incurable lung cancer, severe chronic obstructive lung disease, cardiomyopathy, heart failure or impaired contractility <estimated 40% LVEF or RVEF) 6. Fibrinogen < 2.0 g/L at time of screening 7. Patients considered inappropriate for critical care (prior decision re ceiling of care established) 8. Patients with active bleeding in the preceding 7 days 9. Patients who in the opinion of the investigator are not suitable.
Sample size	36: 12 participants receiving rtPA + standard of care (SOC), and 24 matched historical controls receiving SOC only.
Randomisation	Not applicable – study is open label.
Sponsor protocol number	132151
Funder (s):	Royal Free Charity
EudraCT No	2020-001640-26
ISRCTN / Clinicaltrials.gov no:	NCT04356833

List of Abbreviations

CPAP	Continuous positive airway pressure
CRF	Case report form
IMP	Investigational medicinal product
IMV	Invasive mechanical ventilation
L/RVEF	Left/Right ventricular ejection fraction
NIV	Non-invasive ventilation
PCR	Polymerase chain reaction
rt-PA	Recombinant tissue plasminogen activator
SAE	Serious adverse event
SAP	Statistical analysis plan
SOC	Standard of care
SOFA	Sequential organ failure assessment
WHO	World Health Organization

2. Introduction

2.1 Purpose and scope of the statistical analysis plan

This document describes the main statistical analyses to be applied to the data from PACA Trial. This Statistical Analysis Plan (SAP) was written by Federico Ricciardi (FR) and Aidan O'Keeffe (AOK).

The SAP does not preclude the undertaking of further analyses nor the amendment of any part of the study analysis, should a situation arise in which an amendment or further analysis is deemed necessary. Any changes to the SAP shall be documented and justified.

2.2 Analysis organisation

Unmasking of the data and 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. The primary analysis will be performed independently by two statisticians (FR and AOK) to ensure its accuracy.

2.3 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 and corrected if necessary. All changes will be documented by the trial statistician.

3 Trial Objectives (from Protocol)

3.1 Primary objectives

1. Efficacy: Investigate the potential for efficacy of nebulised rtPA in patients presenting with severe COVID-19 requiring 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 rtPA treatment.

3.2 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 rtPA on other respiratory markers (such as lung compliance) and organ dysfunction;
3. Investigate the impact on in hospital mortality and resource utilisation.

3.3 Exploratory objective

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

4 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 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 rtPA. Patients will be followed up until discharge, death, or day 28 whichever is sooner.

The study will recruit historical controls at a ratio of 2 controls to every 1 rtPA + SOC arm patient, and will be matched according to the following characteristics:

1. Ventilation and oxygen type (IMV and non-invasive oxygen support)
2. Severity as determined by PaO_2/FiO_2 ratio
3. Gender
4. Age (+/- 2 years, up to a maximum of +/- 10 years)
5. Ethnicity

Matching will be done in the order that the characteristics are presented above.

Deceased patients can be considered for matching as controls. However, as a certain number of observations are required for developing a PaO_2/FiO_2 ratio of the curve, patients who died within 2 to 3 days of admission are excluded.

For characteristics 1 and 2, baseline data during admission will be used for matching.

An age range of +/- 2 years was used in the first instance, and this was widened until two eligible patients were identified that matched the treatment patient's ethnicity, up to a maximum of +/- 10 years.

Where more than two controls are identified, two controls will be drawn at random without replacement among all considered historical patients that match the age, gender and severity the case.

4.1 Sample size calculation

The sample size for this pilot study is not based on a statistical consideration. The recruitment target of 12 patients treated with rtPA and SOC and 24 historical controls is based on feasibility and the planned recruitment rate.

4.2 Randomization

Participants in this trial will not be randomized, due to practical and ethical considerations. The first 12 consented patients will receive nebulised rtPA in addition to SOC.

5 Data collection

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.

5.1 Primary outcome measures

Efficacy

- PaO2/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.

Safety

- Incidence and severity of major bleeding events;
- Decrease in fibrinogen levels to < 1.5 gm/L during treatment period and 48 hrs after the last dose of treatment;
- Number and nature of serious adverse events causally related to the treatment.

5.2 Secondary outcome measures

- Changes in lung compliance (defined as tidal volume / (peak inspiratory pressure – PEEP)) from baseline and absolute values at day 5 (96 hrs ± 2 hrs) and day 7 (144 hrs ± 4hrs);
- Clinical status as assessed by a 7-point WHO ordinal scale at baseline and daily up to 5 days post end of treatment and at day 28, discharge or death (whichever comes first);
- Mean daily Sequential Organ Failure Assessment (SOFA) score at baseline through up to 5 days post end of treatment;
- Mean daily NEWS 2 score at baseline through up to 5 days post end of treatment, for NIV patients only;
- In follow up period, number of oxygenation free days, ventilator free days, intensive care stay, up to 28 days or death or discharge, whichever occurs first;

- Incidence and number of days of new oxygen use, non-invasive ventilation or high flow oxygen devices in the first 28 days;
- Incidence and duration of new mechanical ventilation use during in the first 28 days;
- In hospital mortality;
- Length of ICU stay post treatment;
- Length of ventilation;
- Use of vasopressors.

Further details on the outcomes, including a list of references, can be seen in the protocol.

5.3 Exploratory endpoints

Change in 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. Historical controls will not provide data for exploratory endpoints.

5.5 Duration of the intervention period and frequency of follow up

The trial duration per participant is 28 days. Participants will complete assessments at baseline and daily after baseline. Respiratory status of patients will be assessed every 4 hours for the first week, and then daily. The appendix provides a summary of the times at which data are collected.

For historical controls, data will be collected where available for 28 days from the date of admission to hospital. See section 7.5.1 of the protocol for more details.

6 Data analysis plan

6.1 General statistical considerations

All analyses will be descriptive in nature and should be considered as hypothesis generating rather than providing firm conclusions. All statistical tests and confidence intervals will be 2-sided. Significance will be considered at the 5% level and confidence intervals will be at the 95% level.

Analyses for both primary and secondary outcomes will be conducted following an Intention To Treat (ITT) approach, and non-compliance will be reported at the individual patient level. No adjustments for missing data will be made.

6.2 Summary of baseline characteristics

All baseline covariates and baseline valued of the outcomes will be summarised using descriptive statistics. For patients treated with rtPA + SOC, the last available value prior to the first administration of IMP will be considered the baseline value. For historical controls receiving SOC only, the last available value prior to enrolment, as defined in section 8.2, will be considered the baseline value. Categorical variables shall be reported as frequencies and percentages. Reports of continuous variables shall include mean or median and standard deviation or interquartile range (IQR) as appropriate, as well as minimum and maximum values.

Summary measures for the baseline characteristics will be presented both overall and by group. We will compare baseline characteristics visually to assess whether balance has been achieved. No significance testing will be used. The number of missing observations will be reported.

A CONSORT flow chart will be provided (Schulz et al., 2010). This will include the number of eligible participants, number of participants agreeing to enter the trial, then by intervention arm: the number of patients in each group that were enrolled, assessed for eligibility, excluded (with reasons listed), assigned to a group, treated with rtPA, 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).

6.3 Analysis of primary outcome

The primary outcome for efficacy is oxygenation, measured using the PaO2/FiO2 ratio over time. The distributions of the primary outcomes will be summarised, by group, over time. Change in PaO2/FiO2 ratio from baseline will be checked visually using means & SE plots. If necessary, appropriate transformations of the data will be performed (e.g., using the natural logarithm).

Subject to the final sample size and data quality, PaO2/FiO2 ratio will also be compared between the SOC group (historical controls) and rtPA group using appropriate regression modelling, controlling for baseline oxygenation ratio:

$$OXY_i = \beta_0 + \beta_1 \cdot T_i + \beta_2 \cdot OXY0_i + \varepsilon_i,$$

where the *i* subscript denotes the i^{th} patient and

- OXY_i = PaO2/FiO2 ratio (oxygenation) at the end of treatment;
- T_i = group indicator (= 1 if patient is in the rtPA group, = 0 otherwise);
- $OXY0_i$ = PaO2/FiO2 ratio (oxygenation) at baseline;
- $\varepsilon_i \sim N(0, \sigma^2)$ = Normally distributed error term.

The estimate of the parameter β_1 and associated 95% confidence interval shall be reported, as well as a p-value pertaining to the hypothesis test of

$$H_0: \beta_1 = 0 \text{ versus } H_1: \beta_1 \neq 0.$$

Similarly, the use of models using oxygenation at other meaningful timepoints as outcome measure will be evaluated. Owing to a limited sample size, models shall not adjust for other baseline characteristics. Fitted models shall be checked, where appropriate, to ensure that modelling assumptions have been satisfied. Appropriate transformations of outcomes or other suitable approaches (e.g., robust or non-parametric methods) shall be explored if model assumptions are not satisfied.

For treatment safety, the incidence and severity (either major or minor) of bleeding events will be summarised with frequencies and percentages in each group. Fibrinogen levels will also be summarised graphically using plots of the mean, by group, over time. Finally, the number and nature of serious adverse events (SAEs) will be summarised. These events are defined in section 8 of the protocol.

6.4 Analysis of secondary outcomes

All secondary outcomes will be summarized by group. Categorical variables shall be reported as frequencies and percentages. Reports of continuous variables shall include mean or median and standard deviation or interquartile range (IQR) as appropriate, as well as minimum and maximum values.

In addition, we will make use of the daily measurements of clinical status, measurements of lung compliance, NEWS 2 and SOFA score, summarising them at each post baseline timepoint by group. The evolution of these outcomes over time will also be summarised graphically.

7 Software

The statisticians will download the data from the trial specific database into a format suitable to be read by Stata. All the statistical analysis will be performed using Stata version 15 (or above) and/or R version 4.0.2 (or above).

8 References

- Schulz KF, Altman DG, Moher D. (2010). CONSORT 2010 Statement: updated guidelines for reporting parallel group randomised trials. BMC Med, 8(18). doi:10.1186/1741-7015-8-18

Appendix – Schedule of assessment (from Protocol)

	Screening/ 3 day baseline period[a]	During treatment until 5 days after last dose				From 6 days after last dose until day 28/discharge/death	Day 28/discharge/death
		Every 4 hours	Every 6 hours	Once Daily	24 and 48 hours, then twice weekly	Once daily	
Informed consent	X						
Demographics	X						
Review of medical history (including inpatient history and COVID-19 diagnosis)	X						
Eligibility review	X						

Pregnancy test (blood or urine) for female patients of childbearing potential	X						
Review of concomitant medication	X[f]			X		X	X
Review of adverse events/adverse reactions (including bleeding episodes)				X		X	X
Directed physical examination including vital signs (heart rate, temperature, blood pressure and body weight)	X			X		X	X
Documentation of respiratory status[a]	X	X				X	X
Local blood testing[b]	X			X		as required	X
Research blood sampling[c]	X				X	X – weekly	
Administration of rtPA[d]			X				
Clinical status as per 7-point ordinal scale	X			X			X
Sequential Organ Failure Assessment (SOFA) score	X			X			

Glasgow Coma scale	X			X			
End of study data collection [e]							X

[a] Respiratory status of patients is monitored routinely hourly, but data points will be extracted for every four hours where available until 5 days after the last dose (day 7 for SOC patients), but one data point per day is required as a minimum. After this period, respiratory status is to be documented weekly (+/- 1 day) until discharge, death, or 28 days whichever occurs first. See section 7.5 for variables required.

[b] Local blood testing as per section 7.6.2 are to be done at a minimum once a day until 5 days after the last dose, and then as required until day 28, discharge or death whichever occurs first.

[c] Research blood sampling will be done once at baseline at 48 hours (+/- 24 hours), 96 hours (+/- 24 hours), and then twice weekly as per section 7.6.2. Twice weekly testing should be evenly spaced across weekdays if possible.

[d] Dosing schedule as per section 7.3

[e] End of study data will be collected at day 28, discharge or death whichever occurs first as per section 7.5.

[f] to be taken where available once daily for 3 days prior to dosing. Screening assessments can be used as part of this baseline period.