TRIAL STATISTICAL ANALYSIS PLAN (PART 1 ANALYSIS)

c34525499-01

BI Trial No.:	0135-0347
Title:	A Phase IIb/III operationally seamless, open-label, randomised, sequential, parallel-group adaptive study to evaluate the efficacy and safety of daily intravenous alteplase treatment given up to 5 days on top of standard of care (SOC) compared with SOC alone, in patients with acute respiratory distress syndrome (ARDS) triggered by COVID-19.
Investigational Product(s):	Alteplase (recombinant tissue-type plasminogen activator, rt-PA)
Responsible trial statistician(s):	[redacted] Tel.: [redacted] Fax:
Date of statistical analysis plan:	03 May 2021 SIGNED
Version:	Version 2.0

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2. LIST OF ABBREVIATIONS

Term	Definition / description
ACM	All cause mortality
AE	Adverse events
ALT	alanine transaminase
AME	Average marginal effect
AMI	Acute myocardial infarction
ANCOVA	Analysis of Covariance
APACHE	Acute Physiology And Chronic Health Evaluation
ARDS	Acute respiratory distress syndrome
AST	aspartate transaminase
AUC	Area under the curve
BMI	Body mass index
CI	Confidence interval
CRF	Case report form
CRP	Creatinine reactive protein
CTP	Clinical Trial Protocol
CWRES	Conditional weighted residuals
DVT	Deep Vein Thrombosis
ECMO	Extracorporal membrane oxygenation
eGFR	Estimated glomerular filtration rate
EMA	European medicines agency
EU	European union
EudraCT	European Union Drug Regulating Authorities Clinical Trials Database
FAS	Full Analysis Set
GGT	Gamma Glutamyl Transferase
HR	Hazard ratio
ICU	Intensive care unit
INR	International normalised ratio
iPD	Important protocol deviation
IRT	Interactive recognition technology
ISTH	International Society on Thrombosis and Haemostasis

Term	Definition / description
LMWH	Low molecular weight heparin
MBE	Major Bleeding Event
MedDRA	Medical Dictionary for Regulatory Activities
MI	Myocardial infarction
MV	Mechanical Ventilation
NIV	Non invasive Ventilation
PAI-1	Plasminogen Activator Inhibitor Type 1
pcVPC	Predicted corrected visual predictive check
PE	Pulmonary Embolism
PK	Pharmacokinetic
PKS	Pharmacokinetic Set
PPS	Per Protocol Set
PRED	Predications
RBC	Red blood cells
RD	Risk difference
REML	Restricted maximum likelihood
REP	Residual effect period
RPM	Report planning meeting
RS	Randomised Set
rt-PA	recombinant tissue-type plasminogen activator
SCR	Screening Set
SD	Standard deviation
SE	Standard error
SOC	Standard of Care
SOFA	Sequential (sepsis-related) Organ Failure Assessment
TEAE	Treatment emergent adverse events
TPA	Tissue Plasminogen activator
TS	Treated Set
TSAP	Trial Statistical Analysis Plan
UFH	Unfractionated heparin
ULN	Upper limit of normal
VFD	Ventilator Free Days

Term	Definition / description
WBC	White blood cells
WHO	World health organisation

3. INTRODUCTION

As per ICH E9 (1), the purpose of this document is to provide a more technical and detailed elaboration of the principal features of the analysis, as described in the CTP. It will detail the procedures for executing the statistical analysis of the primary and secondary endpoints, and other data, and will be limited to the analysis of Part 1 of BI trial 0135-0347 only.

A database snapshot will be made when all patients have completed the Day 28 visit and when the data has been cleaned. At this point, all analyses outlined in this Trial Statistical Analysis Plan (TSAP) will be performed, except for one, all cause mortality (ACM) at Day 90. This is a further endpoint that will be available at a later timepoint. When this data is available, then another snapshot will be taken and this endpoint analysed.

The results of Part 1 will be used for decision making and for progressing to Part 2, as outlined in the CTP.

An updated TSAP will later be produced to include the handling of the additional data of Part 2.

This TSAP assumes familiarity with the CTP, including Protocol Amendments. In particular, the TSAP is based on the planned analysis specification as written in CTP Section 7 “Statistical Methods and Determination of Sample Size”. Therefore, TSAP readers may consult the CTP for more background information on the study, e.g., on study objectives, study design and population, treatments, definition of measurements and variables, planning of sample size and randomization.

The trial data is stored in the BI Rave (BRAVE) database system.

SAS® Version 9.4 or later will be used for all analyses, except for population PK as outlined in Section 7.8.5.

4. CHANGES IN THE PLANNED ANALYSIS OF THE STUDY

Whilst in the CTP there is a reference to the determination of the Per Protocol Set (PPS), this will only be done for Part 2 of the study. This is not considered to be a change from the protocol, since it will be done for Part 2. This is considered to be a clarification.

Regarding the protocol definition of the primary endpoint, time to clinical improvement or hospital discharge, there has been no detail added regarding patients potentially discontinuing the study early due to withdrawal of consent. In the unlikely event that a patient does discontinue from the study due to withdrawal of consent, prior to being able to determine if he had clinical improvement or hospital discharge, or had intake of bail-out therapy or death, he will be censored at the time of withdrawal. This is not considered to change the protocol definition, rather a clarification.

The randomisation stratification factor baseline ventilation status (Non Invasive Ventilation ((NIV) / Mechanical Ventilation (MV)) is correlated with the baseline status on the world health organisation (WHO) eleven-point clinical progression scale (5, 6, 7, 8 or 9). That is to say, NIV take on values of 5 and 6, whilst MV takes on values of 7, 8 and 9 on the WHO clinical progression scale. Only the randomisation stratification factor ventilation status will be used in the primary model to avoid over parameterising. This is not considered to be a change from the protocol, since this still includes the baseline WHO status.

The secondary endpoint Improvement of Sequential (sepsis-related) Organ Failure Assessment (SOFA) score by ≥2 points from baseline to end of Day 6 is to be determined based upon assessments at Day 6 / hospital discharge. This is not considered to change the protocol definition, rather a clarification.

In Section 6.7 the definition of ‘on treatment’ has been modified, but is not inconsistent with the CTP. That is to say, alteplase ‘on treatment’ starts from first administration of alteplase and ends 288 h later, or longer for patients with more than 5d of infusion. For the SOC group, ‘on treatment’ starts from randomization and ends 288 h later.

Unadjusted estimates of the treatment effect for the primary endpoint, main estimand, will be produced as outlined in Section 7.4.2. For the secondary efficacy endpoints, which are binary in nature, and for the continuous efficacy endpoint number of VFD at Day 28, the unadjusted estimates of the treatment effect will be created in addition. For the continuous efficacy endpoint PaO2/FiO2 ratio at Day 6, the adjusted treatment effect will be created. Details are outlined in Section 7.5.2.

Subgroup analyses not included in the CTP are outlined in Sections 6.7, 7.4.2 and 7.5.2 for the primary endpoint and one of the secondary endpoints.

Any changes to the TSAP from Version 1 to Version 2 are summarized in Section 10.

5. ENDPOINTS(S)

5.1 PRIMARY ENDPOINT(S)

The primary endpoint as defined in Section 2.1.2 of the protocol will be used and will be analysed on the Full analysis set (FAS). This is based on the WHO Clinical Progression scale (10), which ranges from 0 to 10. Clearly, a shorter time to clinical improvement or hospital discharge reflects a better outcome.

> "Time to clinical improvement or hospital discharge up to Day 28, defined as the time from randomisation to either an improvement of two points on the 11-point WHO Clinical Progression Scale or discharge from the hospital, whichever comes first."

However, there are several elements which have to be taken into account, when deriving the endpoint, and in order to address the hypothetical estimand. These are already specified in the protocol and summarized in the table below.

In addition, in the unlikely event that a patient discontinues from the study due to withdrawal of consent, prior to being able to determine if he had clinical improvement or hospital discharge, or had intake of bail-out therapy or death, he will be censored at the time of study withdrawal. This has not been included in the protocol definition but is considered only as a clarification.

Bail-out therapy is as defined in protocol Section 4.2.2.1 Ultima ratio situation - Bail-out, which is 'marketed Actilyse®'. The various scenarios are summarized in the table below:

Table 5.1: 1 Censoring of the primary endpoint for the main estimand

Scenarios	**Main Estimand: Hypothetical situation**
Bail-out then death	Censor at Bail-out
Bail-out then failure to improve#	Censor at Bail-out
Bail-out then clinical improvement / hospital discharge	Censor at Bail-out
Bail-out then clinical improvement / hospital discharge then death	Censor at Bail-out
Clinical improvement / hospital discharge regardless of subsequent events	Positive event at clinical improvement / hospital discharge
Death only, prior to D28	Censor at D28
Failure to improve# by D28	Censor at D28
Discontinued from study due to withdrawal of consent prior to clinical improvement / hospital discharge or Bail-out/Death	Censor at time of withdrawal

Clinical improvement / hospital discharge refers to the first of clinical improvement or hospital discharge, whichever comes first.
#Failure to improve is failure to have clinical improvement or hospital discharge.

The planned analyses of the primary endpoint are outlined in Section 7.4.1, and the supplementary and sensitivity analyses of this endpoint in Section 7.4.2.

5.2 SECONDARY ENDPOINT(S)

5.2.1 Key secondary endpoint(s)

Not applicable.

5.2.2 Secondary endpoint(s)

The secondary endpoints as defined in Section 2.1.3 of the protocol will be used. Efficacy endpoints will be analysed on the FAS, and safety endpoints on the TS. In the case of the secondary endpoints, only Major bleeding event (MBE) up to Day 6 is defined as a safety endpoint.

The planned analyses of the secondary endpoint are outlined in Section 7.5.2.

Improvement of Sequential (sepsis-related) Organ Failure Assessment (SOFA) score by ≥2 points from baseline to end of Day 6, FAS, binary endpoint
The SOFA score is made up of 6 individual components (Pulmonary, Coagulation, Hepatic, Circulatory, Neurologic, Renal), each with an integer value in the range of 0 to 4.
The overall score comprises the sum of the individual components at that visit, the maximum value of which can be 24, and the lower the overall score, the better the health status of the patient. It is to be measured on days 0, 3, 5 or 6, or at hospital discharge if this is prior to day 6.
If the visit was conducted but there are missing components, then the last observation will be carried forward from the previous assessment, in order to determine the overall score. All efforts will be made to have minimal or no missing data.
If the patient does not have a value at Day 6 due to being discharged from hospital prior to this, then it will be considered that the patient had an improvement ≥2 points in SOFA score.
If the patient does not have a value at Day 6 due to death, then it will be considered that the patient did not have an improvement ≥2 points in SOFA score.
If day 6 total value is missing but day 5 total value is available, then day 5 total value will be compared to baseline to check for an improvement of ≥2 points.
Otherwise, SOFA improvement will be left as missing and this patient not included in the denominator.

The overall score will be compared to baseline (Day 0) to see if there is an improvement ≥2 points. Specifically, if value – baseline value ≤ -2 then the patient is considered to have had an improvement ≥2 points in SOFA score at end of Day 6.

MBE (according to International Society on Thrombosis and Haemostasis [International Society on Thrombosis and Haemostasis (ISTH) definition (11)] until Day 6, TS, binary endpoint

If the patient has an MBE, at any timepoint up to end of Day 6 then he has met this unfavorable endpoint. If it is unknown whether the patient had an MBE up to end of Day 6, regardless of the reason, then it will be assumed that the patient did not have an MBE by the end of Day 6. This is a safety defined endpoint and the starting point for consideration should be consistent with the 'on treatment' definition in Section 6.7, however only including events up to midnight of Day 6.

ACM at Day 28, FAS, binary endpoint

If it is unknown whether the patient was dead at end of Day 28, then it will be assumed that the patient did not die up to Day 28, regardless of the reason. This unfavorable endpoint is met if:

- the last known status of the patient is 10 on the WHO clinical progression scale by the end of Day 28, or
- vital status is dead within 28 days

ACM / MV at Day 28, FAS, binary endpoint

If it is unknown whether the patient was on MV at Day 28, it will be assumed that the patient was not on MV at Day 28, regardless of the reason. This unfavorable endpoint is met if:

- the last known status of the patient has a value of ≥7 on the WHO clinical progression scale, up to end of Day 28, or
- vital status is dead within 28 days

Daily average PaO2/FiO2 ratio (or inferred PaO2/FiO2 ratio from SpO2) change from baseline to Day 6, FAS, continuous endpoint

This assessment is to be measured approximately 3-times daily on each of days 0 to 7, and again on day 28, but only whilst the patients is still in hospital. All available values on each of these days, regardless of the position of the patient when being measured, will be averaged in order to determine the daily average PaO2/FiO2 ratio for that patient. The higher the value the better the health status of the patient.

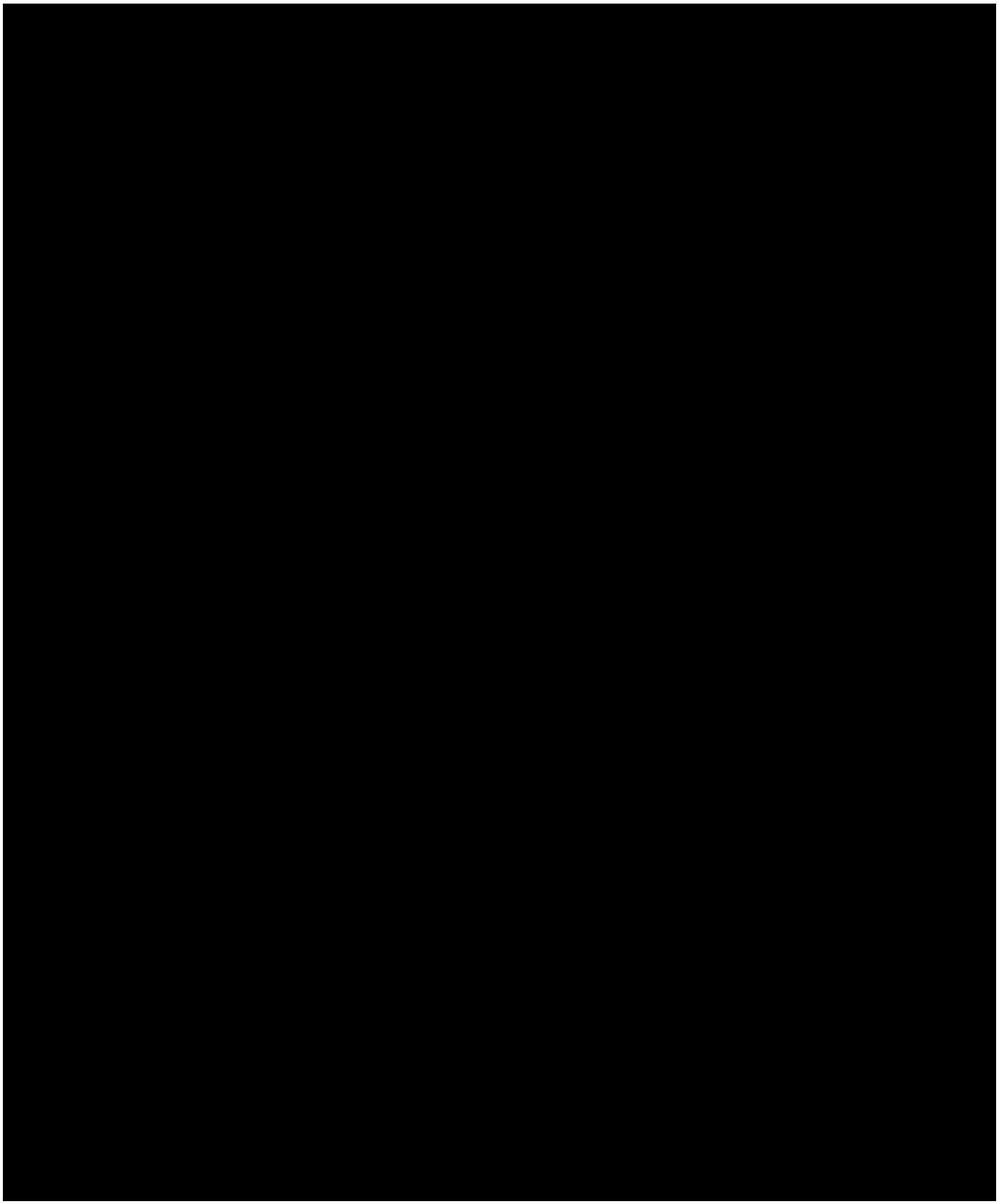
- If the patient is still in hospital during day 6 then the day 6 daily average value will be used, if available.
- If the patient has been discharged from hospital prior to day 6 then the daily average at the time of hospital discharge will be used as a surrogate for day 6, if available.
- If the patient has died prior to day 6 then there will be no imputation but the death handled as failure in the determination of the difference in medians and 95% CI.

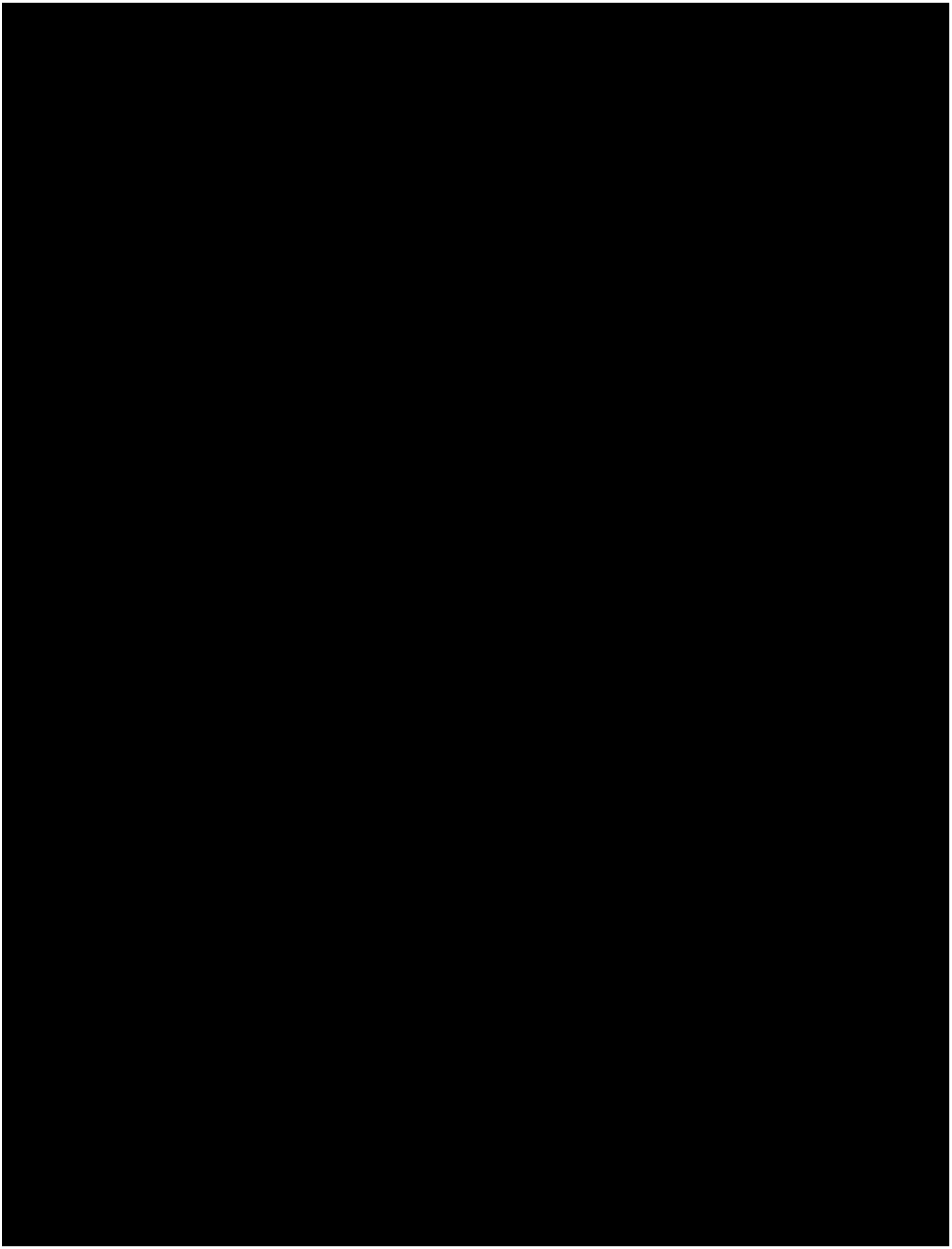
Based upon this, the change from baseline for each patient will be calculated and used for the analysis.

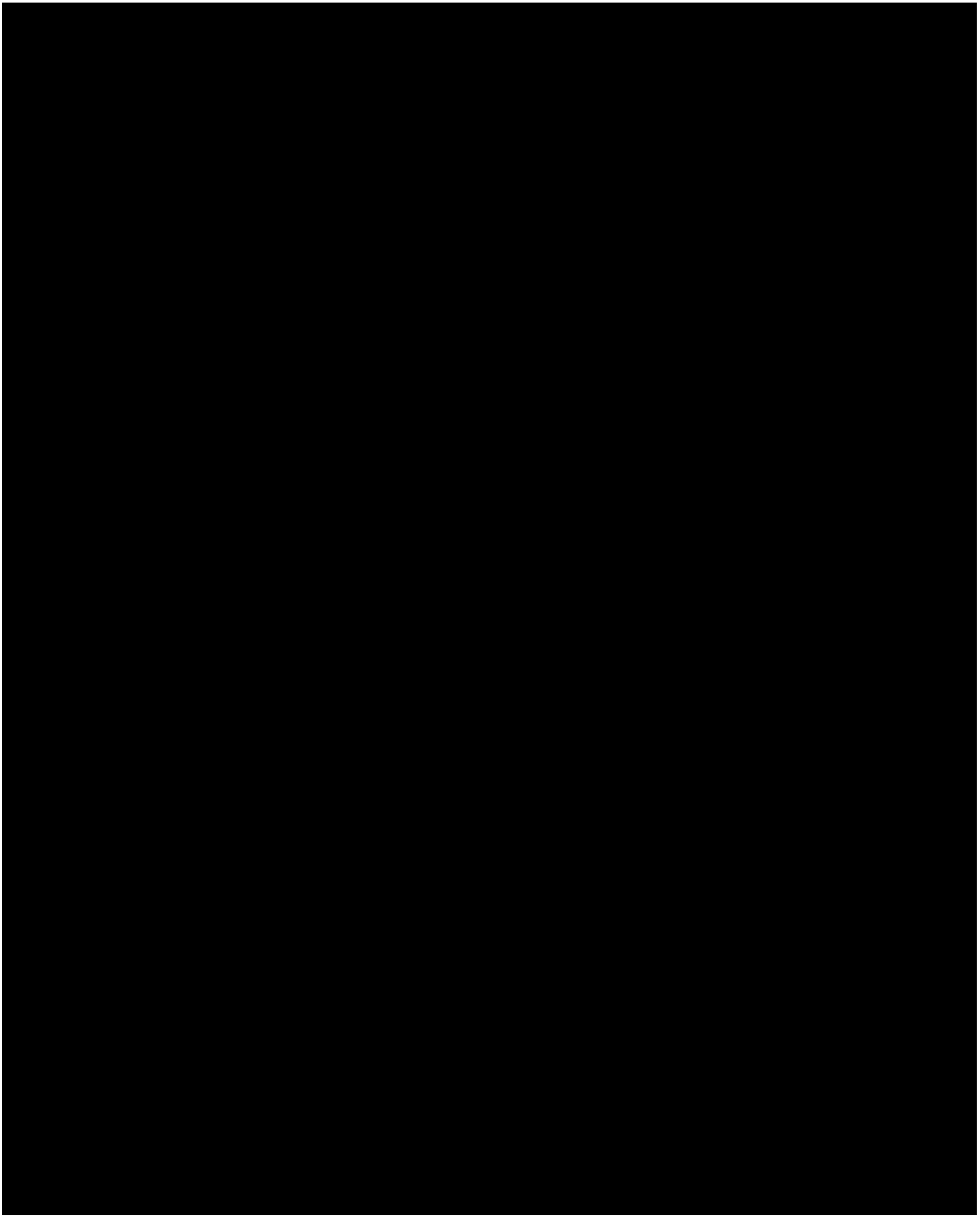
Number of ventilator-free days (VFD) from start of treatment to Day 28, FAS, continuous endpoint

For this endpoint, 'ventilator' is defined as 'assisted breathing' but it refers to mechanical invasive ventilation. The number of VFDs starts from when the patient has a 'lasting' value on the WHO clinical progression scale of ≤ 6, and ends on Day 28. A lasting value of ≤ 6 means that the value cannot exceed 6 at a later timepoint. If the patient is liberated from the

ventilator on Day x, then the number of VFDs is 28-x. If a patient has withdrawn consent prior to day 28 then he will have a missing value for VFD. In any case, if the status of the patient at Day 28 is death, as determined from the vital status page then the VFD=0.







6. GENERAL ANALYSIS DEFINITIONS

6.1 TREATMENT(S)

For the purposes of the statistical analyses, treatments will be referred to in the same context as in Section 7.4 of the protocol.

- "Alteplase low", which is alteplase 0.3mg/kg over 2 hours followed by 0.02 mg/kg/h over 12 hours
- "Alteplase high", which is alteplase 0.6 mg/kg over 2 hours followed by 0.04 mg/kg/h over12 hours
- Standard of Care (SOC)

Alteplase treatment is planned to be up to end of Day 5 but all patients should be taking SOC in the background. On day 1 there is an initial 2 h infusion for all, followed up by a longterm 12 h infusion. On days 2-5 the patients can receive an optional 2h infusion (based on investigator decision), but only on one of these days. Each patient should receive a longterm 12h infusion on these remaining 4 days.

6.2 IMPORTANT PROTOCOL DEVIATIONS

Whilst is it not planned to conduct a Per Protocol analysis for Part 1 of this study (too few patients), patients with any of the following important protocol deviations (iPDs) will be reviewed on a case by case basis (2) and (3). The review of the iPDs will be used to determine criteria for a PPS for Part 2 of the study.

Note that this is a working list and may be updated again prior to the final Report Planning Meeting (RPM) prior to the snapshot related to end of Part 1. Inclusion criteria/exclusion criteria numbers refer to the definition given in the protocol or CRF.

Table 6.2: 1 Handling of iPDs

iPD code	iPD Category & Brief Description
A	**Entrance Criteria Not Met**
A1	**Inclusion Criteria not met**
A1.1	Patient too young (IC1)
A1.2	Diagnosis of ARDS questionable (IC2,4,5)
A1.3	Patient does not test positive for SARS-CoV-2 (IC3)
A2	**Exclusion Criteria not met**
A2.1	Patient has baseline conditions that are not permitted for safety reasons (EC1-31)

Table 6.2: 1 Handling of iPDs (continued)

A2.2	Patient has baseline conditions that prevent patient from complying with study protocol (EC1-31)
A2.3	Patient has had previous conditions or procedures that are not permitted (EC1-31)
A2.4	Patient has had forbidden previous therapy (EC1-31)
A2.5	Patient pregnant (EC25)
B	**Informed Consent**
B1	Informed consent not available (IC6)
B2	Informed consent not signed at screening visit 1 (IC6)
B3	Informed consent not available or not available at visit 2a (pre-dose) (IC6)
C	**Trial medication and randomisation**
C1	Method of assigning patients to treatment arms
C1.1	Randomisation not followed according to protocol
C1.2	Patients do not receive the initial treatment they were randomised / allocated to
C1.3	Patient assignment not followed
C1.4	Timeframe > 1 day between randomisation and first drug intake
C2	**Non-compliance**
C2.1	Overall Compliance not between 80% and 120% inclusive – see definition in Section 5.4.
C2.2	Patient takes the optional 2h infusion on more than one occasion after the initial 2 h infusion.
C3	**Drug assignment and administration of doses for each patient**
C3.1	Dosage and treatment schedule not given according to protocol for initial iv infusion
C3.2	Dosage and treatment schedule not given according to protocol for long term iv infusion
C3.3	Drug not permanently discontinued according to Section 3.3.4.1 of CTP
C3.4	Drug not temporarily interrupted according to Section 3.3.4.1 of CTP

Table 6.2: 1 Handling of iPDs (continued)

D	**Concomitant medication**
D1	Intake of restricted concomitant medication according to protocol section 4.2.2.1 and Section 9.2
D2	Intake of marketed Actilyse® (non-study drug) not as ultima-ratio or bail-out
G	**Other trial specific important deviations**
G1	Other protocol violations affecting patient rights or safety (manual PDs to be captured)

6.3 SUBJECT SETS ANALYSED

The subject sets as defined in protocol Section 7 for the Randomised Set (RS), Full Analysis Set (FAS) and Treated Set (TS) will be used.

The Screened set (SCR), not outlined in the CTP, will include all patients screened.

The RS will consist of all randomised patients.

The FAS will consist of all randomised patients with at least one baseline and one post baseline assessment relating to the primary endpoint of interest. That is to say, they need to have at least one assessment post baseline on the 11-point WHO Clinical Progression Scale.

The TS will consist of all patients who were randomised and, for patients in the alteplase groups, treated with at least one dose of trial drug.

The Pharmacokinetic set (PKS), not outlined in protocol, will comprise all subjects in the TS who provided at least one PK endpoint and had no important protocol violations relevant to the evaluation of PK.

Table 6.3: 1 Subject sets analysed

	Subject set			
Class of endpoint	**SCR / RS**	**TS**	**FAS**	**PKS**
Primary endpoint			primary and supplementary analyses	
Secondary and further EFFICACY endpoints			X	
Secondary and further SAFETY endpoints		X		
Treatment exposure		X		
Disposition	X			
Demographic/baseline		X	X	
Concomitant medications and compliance		X	X	
All other safety analyses		X		
PK analyses				X

6.5 POOLING OF CENTRES

Part 1 of this study is planned to be carried out in approximately 35 centres. However, not all centres have the same SOC procedures. During the course of the study, and gathering information (retrospectively) from each of the centres with regard to their SOC procedures, they will be grouped into relatively homogeneous groupings.

Because the groupings will only be made after randomisation of a patient within a centre, this will be a retrospective and exploratory analysis, albeit planned. It is planned only to investigate the primary endpoint with this centre grouping in a sensitivity analysis – See Section 7.4.2.

6.6 HANDLING OF MISSING DATA AND OUTLIERS

All efforts will be made to avoid having missing data in the database, however it is inevitable that there will be missing data, by design:

- For patients that die, there will be no further data.
- For patients that are discharged from hospital before day 28, the only additional efficacy assessment is the vital status at days 28 and 90.
- For the daily average PaO2/FiO2 ratio at Day 6: if no value available due to hospital discharge, then use value at hospital discharge; if no value available due to death then do not impute but handle as failure in analysis to determine difference in median with 95% CI. Otherwise, outcome unknown and not included in denominator.

- For SOFA improvement: if no value available due to hospital discharge the improvement=yes; if no value available due to death then improvement=No; if missing components but assessment conducted, the previous components can be carried forward; if day 5 value available then this can be used in place of day 6; then improvement from baseline determined. Otherwise, outcome unknown and not included in denominator.
- For Evolution of APACHE II and daily average PaO2/FiO2: carry forward missing APACHE II components if possible (APACHE II); otherwise no imputation but present the number of cumulative hospital discharges and cumulative deaths in the same table.

Missing or incomplete adverse event (AE) dates will be computed using the BI handling rules (4).

6.7 BASELINE, TIME WINDOWS AND CALCULATED VISITS

Randomisation is defined as Day 1 and it is planned that the alteplase patients are administered their initial 2h infusion of study medication within 6 hours of randomisation, on Day 1 (Visit 2a). The last planned administration of study medication for the alteplase patients is the long term 12h infusion on Day 5.

Since all patients are on SOC and there is no clear start or stop of SOC, a fixed treatment duration will be used for all patients, for the purposes of the analyses. Therefore, endpoints referred to as ‘on treatment’ will include all relevant events from the relevant start point plus 288 hours (last planned treatment on Day 5 + 7 days of REP = 12 days = 288 hrs). The starting point for the alteplase patients is date and time of first administration of alteplase, whilst the starting point for the SOC patients is the date and time of randomisation. For alteplase patients which have more than 5 days of infusion, the end date of ‘on treatment’ will be the date and time of last intake of alteplase plus 168h (=7 days).

According to CTP Section 1.2, the residual effect period (REP) is 7 days.

According to CTP Section 7.2.1, the term "baseline" refers to the last observed measurement prior to randomisation on Day 1, which will be Visit 1, the screening visit. It cannot be determined with certainty if a value measured on Day 1 is prior to randomisation, so the screening value will be used consistently throughout. Only if otherwise indicated, will the assessment on Day 1 be used, where the time of the assessment can be compared to the time of the randomisation.

7. PLANNED ANALYSIS

The tables that are created from the statistical analysis will follow the format of the standard within BI (5).

The overall disposition of the patients in the trial will be presented, together with the number of patients discontinuing prior to day 28, and vital status at day 90.

The number of patients participating in the study by country and the number of sites, for each of the patient populations will be summarised.

Demography and baseline characteristics will be presented, as well as the stratification used at the time of the patient randomisation.

A summary of the iPDs will be provided.

Where statistical comparisons are indicated, they will be done by comparing the following groups:

- alteplase low versus SOC
- alteplase high versus SOC

For efficacy analyses, patients will be analysed according to the patient information (ventilation status) given in the CRF (NIV / MV), in the case of potentially erroneous data entered into the interactive recognition technology (IRT).

In general, for End-Of-Text tables, the set of summary statistics is: N (number of patients with non-missing values) / Mean / SD / standard error (SE) / Min / Q1 (lower quartile)/ Median / Q3 (upper quartile)/ Max.

Statistical parameters will be displayed to a defined number of decimal places as specified in the BI guideline "Reporting of Clinical Trials and Project Summaries" (6).

Tabulations of frequencies for categorical data will include all possible categories and will display the number of observations in a category as well as the percentage (%) relative to the respective treatment group (unless otherwise specified, all patients in the respective patient set whether they have non-missing values or not). Percentages will be rounded to one decimal place. The category missing will be displayed only if there are actually missing values.

7.1 DEMOGRAPHIC AND OTHER BASELINE CHARACTERISTICS

Only descriptive statistics are planned for this section of the report. See Section 5.4 for specific definitions.

7.2 CONCOMITANT DISEASES AND MEDICATION

Medical history, COVID-19 signs and symptoms and concomitant diagnoses will be presented and coded using MeDRA System Organ Class and Preferred Term where possible.

Regarding concomitant medications, there is a specific question asking if the treatment belongs to standard of care, which is relevant for all patients since they all receive SOC in the background. This question is different to the randomised treatment assignment. Therefore, all concomitant medication analyses or displays will be split by 'Standard of Care use' and 'non-Standard of Care use'. All concomitant medications will be coded using the WHO Drug dictionary.

Medications of special interest for COVID (as indicated by WHO, see Section 9.1), and restricted medications within this study (as outlined in CTP Section 4.2.2.1, see Section 9.2) will also be summarized and split by Standard of Care use and non-Standard of Care use.

Use of heparin will be summarized and presented.

Therefore, only descriptive statistics are planned for this section of the report.

7.3 TREATMENT COMPLIANCE

Only descriptive statistics are planned for this section of the report, and due to the nature of the study, compliance will be determined for the alteplase treatment groups only

Compliance to intake of infusion will be assessed in terms of % taken, as measured by volume, at each planned infusion. Overall compliance will be as an average measure of the %taken. The detailed definitions are provided in Section 5.4.

Compliance with preparation of the infusion in terms of concentration, as indicated in CTP Table 10.5 are also assessed, and detailed definitions provided in Section 5.4.

7.4 PRIMARY ENDPOINT(S)

7.4.1 Primary analysis of the primary endpoint(s)

For the primary endpoint, a cox proportional hazards model will be used to estimate the hazard ratio (HR) comparing the alteplase groups to SOC. From the model, the 95% confidence intervals (CI) and corresponding Wald p-values for the HRs will be produced. The model will include fixed effects for treatment (alteplase group of interest, SOC), the randomisation stratification factor type of ventilation (NIV, MV) and age (continuous).

Baseline ventilation status (NIV/MV) and baseline status on the WHO eleven-point clinical progression scale (5, 6, 7, 8 or 9) are correlated. That is to say, NIV take on values of 5 and 6, whilst MV takes on values of 7, 8 and 9 on the WHO clinical progression scale. Of these two, only the randomisation stratification factor ventilation status will be used in the primary model to avoid over parameterising. This is not considered to be a change from the CTP, since this still includes the baseline WHO status.

Kaplan Meier estimates will be presented by treatment (low alteplase, high alteplase, SOC) for this endpoint up to Day 28, and additionally by:

- treatment and type of ventilation (NIV, MV)
- treatment and baseline status on the WHO eleven-point clinical progression scale (5, 6, 7, 8 or 9)
- treatment and age groups (<=median, >median)

From the KM estimates stratified by treatment, and at various timepoints (days 6, 8, 12, 16, 20, 24 and 28) the risk difference (RD) with 95% CI will be estimated by taking the difference of the individual probabilities. Since the observations in the two treatment groups are independent, the variance of the Kaplan-Meier probability difference is equal to the sum of the Kaplan Meier probabilities' variances found by Greenwood's method. The 95% CI of the RD is therefore calculated accordingly.

7.4.2 Sensitivity analysis, subgroup analysis, exploratory analysis of the primary endpoint(s)

Two non-confirmatory supplementary analyses will be conducted, in anticipation of the rare situation where bail-out therapy may be used (as defined in CTP Section 4.2.2). They will be performed on the FAS.

The first of these supplementary analyses will address the treatment policy estimand, whereby there is no consideration for the intake of bail-out therapy.

The second of these supplementary analyses will address a composite estimand, whereby patients receiving bail-out therapy will be censored at Day 28, rather than on the day of bail-out. Here, bail-out represents treatment failure and thereby, by definition, precludes any response after bail-out. The various scenarios are summarized in the table below.

In both cases, and in the unlikely event that a patient discontinues from the study, prior to being able to determine if he had clinical improvement or hospital discharge, or had intake of bail-out therapy or death, he will be censored at the time of withdrawal.

Table 7.4.2: 1 Censoring of the primary endpoint for the treatment policy and composite estimands

Scenarios	**Treatment policy estimand**	**Composite estimand**
Bail-out then death	Censor at D28	Censor at D28
Bail-out then failure to improve#	Censor at D28	Censor at D28
Bail-out then clinical improvement / hospital discharge	Positive event at clinical improvement / hospital discharge	Censor at D28
Bail-out then clinical improvement / hospital discharge then death	Positive event at clinical improvement / hospital discharge	Censor at D28
Clinical improvement / hospital discharge regardless of subsequent events	Positive event at clinical improvement / hospital discharge	Positive event at clinical improvement / hospital discharge
Death only, prior to D28	Censor at D28	Censor at D28
Failure to improve# by D28	Censor at D28	Censor at D28
Discontinued from study due to withdrawal of consent prior to clinical improvement / hospital discharge or Bail-out/Death	Censor at time of study discontinuation	Censor at time of withdrawal

Clinical improvement / hospital discharge refers to the first of clinical improvement or hospital discharge, whichever comes first.
#Failure to improve is failure to have clinical improvement or hospital discharge.

The analyses performed will be identical to that outlined for the primary endpoint in Section 7.4.1.

Based on the main estimand, the treatment effect will be estimated using the cox proportional hazard model, containing only treatment in the model. The HR (95%CI) and p-value estimating the unadjusted treatment effect will be produced.

The effect of the subgroups outlined in Section 6.4, related to the primary endpoint, main estimand, will be assessed descriptively and using Kaplan Meier estimates.

A covariate adjusted sensitivity analysis of the primary endpoint, main estimand, will be conducted, to determine the effect of the grouping of centres according to similar types of Standard of care. This will be the same analysis as outlined in Section 7.4.1 with this additional covariate.

7.5 SECONDARY ENDPOINT(S)

7.5.1 Key secondary endpoint(s)

Not applicable.

7.5.2 Secondary endpoint(s)

Secondary endpoints are defined in Section 5.2.2. In all cases, standard descriptive statistics will be presented, whether the endpoint is binary or continuous.

Binary efficacy endpoints
For the binary efficacy endpoints (ACM, ACM/MV, SOFA), analysed on the FAS, the RD will be estimated between the two treatment arms using logistic regression. In addition to treatment, adjustment will be for the randomization stratification factor (NIV/MV) and age. RD and CIs (95%) for the RD will be obtained using the Delta method and the average marginal effect (AME) method.

As sensitivity analysis for each of these binary efficacy endpoints, the unadjusted treatment estimates will be produced, using the same logistic regression model containing only treatment.

Binary safety endpoint
For the binary safety endpoint MBE, analysed on the TS, there will be no adjustment for covariates. Therefore, the RD will be the difference between the two incidences and the 95% CI will be determined using the Chan and Zhang exact method.

Continuous efficacy endpoint
The change from baseline in the continuous efficacy endpoint, daily average PaO2/FiO2 ratio at Day 6 is considered non-parametric in nature. Missing values for missing visits will not be imputed, and left as missing. Missing values due to death or post hospital discharge will be carried out as described in Section 5.2.2. The difference in medians will be presented, together with 95% CIs, based upon the methodology of Hodges-Lehmann and the Wilcoxon rank sum test. There will be no adjustment for covariates in this non-parametric analysis.

As a sensitivity analysis for this PaO2/FiO2 endpoint, an ANCOVA analysis adjusting for baseline ventilation status and age will be performed. For patients that have died, the last value prior to death will be used to determine the change from baseline. Note, there are expected to be very few deaths prior to Day 6. Missing values due to hospital discharge will be handled as in the main analysis.

For the continuous efficacy endpoint, number of VFDs at Day 28, there are not expected to be any missing values by definition. This endpoint will be analysed using a restricted maximum likelihood (REML) based ANCOVA comparing the number of VFDs after 28 days, by treatment. The ANCOVA model will be adjusted for the randomization stratification factor (NIV/MV) and age. The treatment difference with 95% CI will be presented.

As a sensitivity analysis for VFD at Day 28, the unadjusted treatment effect will be estimated, using the same approach as the main analysis, but with only treatment in the model. In addition, subgroup analyses of VFD at Day 28 will be conducted using the subgroups as outlined in Section 6.4. An ANCOVA model will contain the subgroup and the interaction between subgroup and treatment.

7.7 EXTENT OF EXPOSURE

Exposure will be handled descriptively.

The number of patients with initial 2h infusion on day 1, optional 2h between days 2 and 5, and longterm 12h infusion over the five days of treatment will be summarized, as well as the rate of infusion (mL/kg/h) and duration of infusion (h).

The cumulative exposure will be presented, in terms of Total number of days of infusion. Exposure is relevant for the alteplase treatment groups only.

7.8 SAFETY ANALYSIS

The safety analysis will be performed as outlined in the CTP, according to BI standards and on the TS. For further details please see guideline 'Handling and summarization of AE data for clinical trial reports and integrated summaries' (7).

The definition of ‘on treatment’ is outlined in Section 6.7. Any potential events starting between the time of randomization and the start of alteplase treatment, will be indicated as such in the listings.

In addition, to fulfil the requirements of the European Union (EU) Regulation 536/2014 (Annex V, Point 6) (8), the following information should be made available, for the lay summary or CTR:

- frequency of serious drug-related AEs by treatment, primary system organ class and preferred term.

7.8.1 Adverse Events

Unless otherwise specified, the analyses of AE will be descriptive in nature. All analyses of AEs will be based on the number of subjects with AEs and NOT on the number of AEs.

AEs will be coded using the most recent version of the Medical Dictionary for Regulatory Activities (MedDRA) coding dictionary

The analysis of AEs will be based on the concept of treatment emergent adverse events (TEAE). That means that all adverse events occurring 'on treatment' as outlined in Section 6.7 will be considered as TEAE.

All AEs occurring before 'on treatment' will be assigned to 'screening' and all AEs occurring after 'on treatment' will be assigned to 'post-treatment'. In general, AEs attributed to 'Screening' or 'Post-treatment' will be listed only.

An overall summary of AEs will be presented.

Frequencies [N (%)] of patients with AEs will be summarised by treatment, primary system organ class and preferred term (using MedDRA). Separate tables will be provided for patients with serious adverse events.

The system organ classes will be sorted according to the standard sort order specified by European medicines agency (EMA), preferred terms will be sorted by frequency (within system organ class). The severity of AEs will be summarised by the maximum intensity of the events each patient had. This will show the number and percent of patients who had at most mild, moderate, severe, and life threatening events. Severe AEs is defined as an AE with mild, moderate, severe grade.

Additionally, the following analyses will be reported for disclosure on European Union Drug Regulating Authorities Clinical Trials Database (EudraCT) and clinicaltrials.gov:

- Frequency [N(%)] of patients with non-serious AEs occurring with incidence in preferred term greater than 5% by treatment,
- AEs per arm for disclosure on EudraCT by treatment
- Non-serious AEs for disclosure on EudraCT by treatment
- Serious AEs for disclosure on EudraCT by treatment

Adverse events of interest
All endpoints related to safety, outlined in Section 5.2.2 and Section 5.3, are clearly marked as such. In particular, endpoints related to MBE, Stroke, MI, PE and DVT. There is no further description below on how they should be analysed, however, they will be summarized with the safety data under the topic of 'AEs of special interest'.

There are two other AEs of special interest, which are not mentioned in Sections 5.2.2 and 5.3:

- Transient ischemic attack
- Systemic embolism

AEs recorded on the AE page of the CRF, indicated as such via the relevant tick box, will be grouped and summarized using frequency tables.

7.8.2 Laboratory data

Changes in coagulation and inflammatory markers on treatment, as outlined in Section 5.3, are the laboratory parameters of special interest in this trial. They will be analysed in the efficacy section.

The analyses of laboratory data will be descriptive in nature and will be based on BI standards "Handling, Display and Analysis of Laboratory Data" (9).

The following laboratory parameters are considered safety and hence processed according to standard safety processing rules:

- Haematology (Haematocrit, Haemoglobin, Red blood cells (RBC) / Erythrocytes, White Blood Cells (WBC) / Leukocytes, Platelet Count / Thrombocytes)

- Clinical Chemistry (Albumin, Alkaline phosphatase, ALT (alanine transaminase), AST (aspartate transaminase), GGT (Gamma Glutamyl Transferase), Bilirubin total, Bilirubin direct, Creatinine, eGFR, Creatine kinase, Troponin, Lactate dehydrogenase, Glucose, Potassium, Sodium, Calciom, Magnesium, Urea (BUN), NT-proBNP, Cystatin C, Total Cholesterol, LDL- cholesterol, HDL- cholesterol, Triglycerides, Protein total, Glucose, Hba1c, C-peptide.

For continuous safety laboratory parameters standardized values will be derived as well as the differences to baseline. Laboratory parameters will be shown in SI units.

Laboratory values will be compared to their reference ranges and frequency tables will be provided for the number of patients within and outside the reference range at baseline, each visit and the last measurement on treatment. Descriptive statistics will be provided by treatment group for baseline, each visit and last value on-treatment and for changes from baseline to each respective visit and last value on treatment. The laboratory parameters will be summarized by day of assessment.

7.8.3 Vital signs

The following vital signs will be descriptively analysed: systolic and diastolic blood pressure, body temperature, respiratory rate and pulse rate. Both absolute values and change from baseline are planned for this section of the report.

7.8.4 ECG

Not applicable.

7.8.5 Others

Pharmacokinetic analysis

Descriptive statistics of all PK parameters will be presented and will be performed on the PKS as outlined in Section 6.3. In particular, the parameters / endpoints are:

- Css,1 (plasma concentration of alteplase at steady state of 2 hour infusion)
- Css,2 (plasma concentration of alteplase at steady state of long-term infusion)

Individual endogenous Tissue Plasminogen activator (TPA) will be determined for each patient at baseline using the first PK sample. Baseline levels will then be subtracted from all further concentration measurements (second and third samples), for each patient, in order to determine the Css,1 and Css,2 endpoints. In case the resulting concentration value is negative, it will then be set to "BLQ".

As outlined in CTP Section 7.2.4, a population PK analysis is planned to be conducted if sufficient data are available. The objectives of the population PK analysis are:-

1) to compare the alteplase concentrations in study 0135-0347 to PK simulations based on a previously developed population PK model for patients with acute myocardial infarction (AMI)
2) to derive model-based area under the curve (AUC) estimates for patients with ARDS based on the sparse PK measurements of study 0135-0347

Dataset preparation
Alteplase plasma concentrations, sampling times, infusion times, and covariates (e.g., age, weight, sex, and race) will be assembled and formatted for analysis. Detailed dataset specifications will be provided in a separate document.

Handling of missing data
Handling of missing data will be described in the dataset specification document.

Handling of outliers
Alteplase concentrations and covariate values will be included in the analysis whenever possible. However, for methods based on least-squares estimation and normal theory it is not good practice to include extreme values. Thus, outlying data points in the dependent variable may be excluded if they are infrequent, occur randomly and are spurious. Furthermore, it may be justified to exclude outlying individuals from model development. Also, extreme covariate values might be excluded. If more than 5% of alteplase plasma concentrations are excluded, a sensitivity analysis will be performed to evaluate the impact of the excluded data points on the final model. All outliers which are excluded from the analysis will be documented along with reasons for their exclusion.

Pharmacokinetic modeling strategy

In the following the framework of the PK modelling strategy is outlined, this may be adjusted as deemed necessary.

The pharmacokinetics of alteplase follows a 3-compartment distribution. A 3-compartment model cannot be developed using the information from study 0135-0347 alone as only sparse PK data is collected in a subset of the study population. Therefore, a previously developed population PK model for alteplase will be used to inform the current analysis. The population PK model for patients with AMI which is based on studies 0135.30 and 0135.53 is the preferred starting model. The pharmacokinetics of alteplase in these AMI studies were best described by a linear 3 compartment model. A baseline t-PA concentration was estimated to account for endogenous t-PA in plasma. Weight was included as covariate affecting baseline t-PA concentration and clearance of alteplase. Age was included as covariate affecting clearance of alteplase.

First, the alteplase plasma concentrations in study 0135-0347 will be compared to PK model simulations. Simulations will be performed using the previously developed population PK model for alteplase in patients with AMI. In the simulations, inter-individual and residual variability will be considered. The alteplase plasma concentration-time profiles will be simulated 1,000 times for each subject in study 0135-0347 assuming its actual infusion regimen, sampling scheme and weight. The simulated alteplase plasma concentrations at the different time points will be used to calculate the median concentration-time profile as well as it's 90% prediction interval. This simulation results will be graphically compared to the observed data in study 0135-0347.

Given that the model-simulated plasma PK is in the same range as the observations, it will be further investigated whether the population PK model for patients with AMI can describe the individual alteplase concentrations in ARDS patients. Individual post-hoc estimates will be derived for study 0135-0347 using the parameters of the population PK model for patients with AMI. The distribution of the post-hoc estimates around the typical parameters and the description of the individual plasma concentrations will be investigated.

Provided that the population PK model for patients with AMI successfully describes the individual profiles of patients with ARDS, the individual PK parameters will be determined. In addition, AUC will be derived within NONMEM® from the individual PK parameters considering the patients' actual alteplase doses, infusion regimen, and covariates. In case, the structural PK model of patients with AMI cannot describe the PK of patients with ARDS, necessary adjustments (e.g. re-estimation of clearance) might be implemented into the model.

The population PK model for patients with AMI comprises the impact of weight and age on the PK of alteplase. Assessment of any remaining trends for weight, age, sex, and race will be conducted e.g. by graphical inspection plotting empirical Bayes estimate versus covariate values and conditional weighted residuals (CWRES) versus covariate values.

Inter-subject variability will be modeled using exponential random effect models. Residual variability will be modeled starting with the same error model structure as in patients with

AMI; adjustments will be made if necessary. The set of inter individual (η) and residual (ε) variability values are assumed to be symmetrically distributed around 0 with variances ω^2 and σ^2, respectively.

Evaluation

If model development is necessary, objective function values (for nested models) as well as the criteria listed below will be considered:

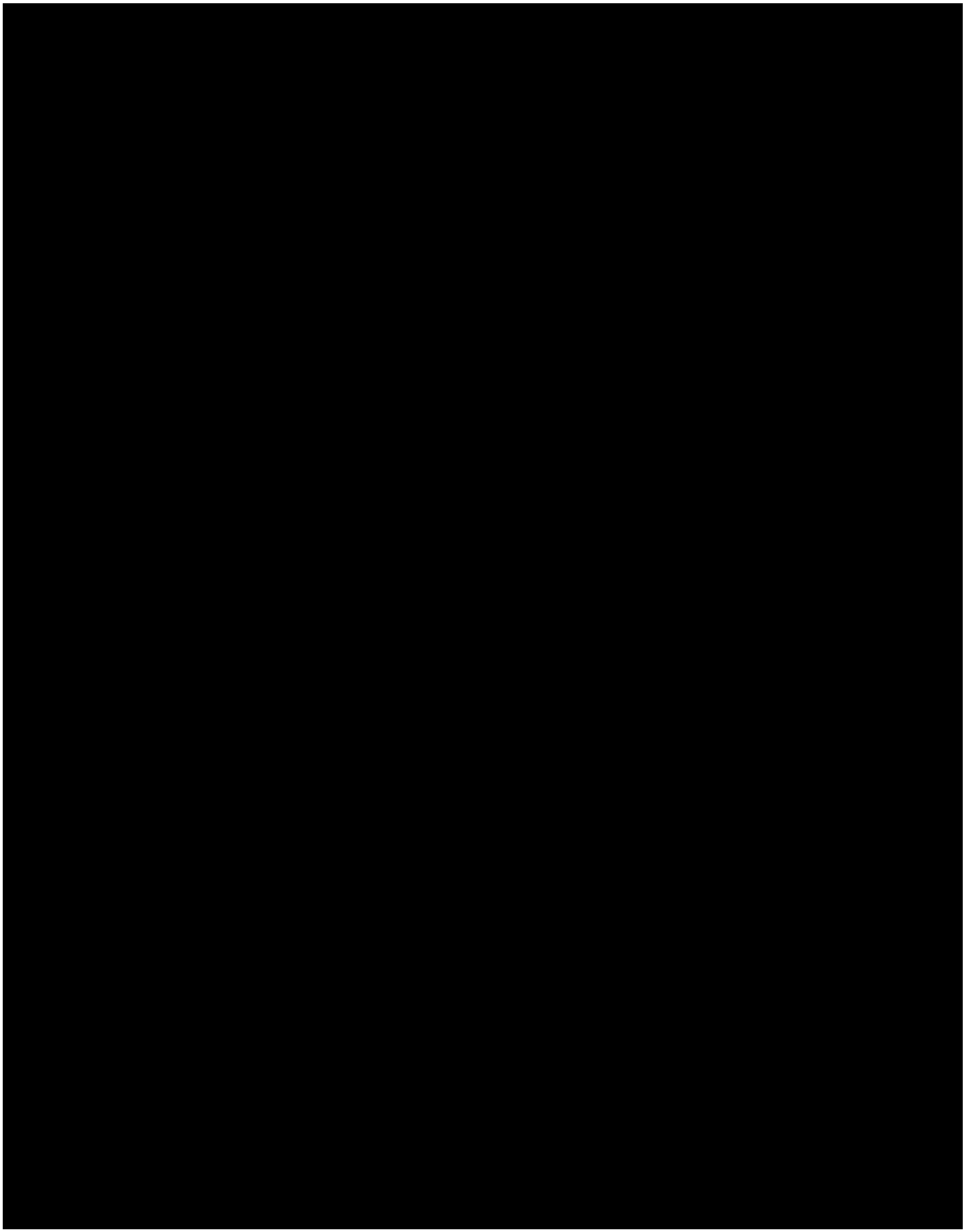
- A "successful minimization" statement by the NONMEM® program at the end of the estimation step.
- The final parameter estimates have a number of significant digits of 3 for all θ's.
- Estimates of θ's not close to a boundary.
- Correlation of the uncertainty of the estimates of fixed effect parameters < 0.95 or >-0.95.
- No significant trends in the basic goodness-of-fit plots (predictions (PRED), individual predictions (IPRED) vs. DV)
- Residuals (CWRES) scatter randomly and uniformly around zero when plotted against population prediction and time.
- ETAs are normally distributed around 0.
- No significant model misspecification in the individual predicted concentration-time profiles when compared to the observed profiles.

If a model is accepted as final model, although one or more of the criteria above are not fulfilled, the reasons for its acceptance need to be given in the report.

If model development is necessary, the final model will be evaluated using a prediction corrected visual predictive check (pcVPC, 12). A visual predictive check shows the ability of the model to simulate data that are similar to the observed data that were used for model development. The concentration-time profiles will be simulated 1,000 times using the same actual doses, infusion regimen, subjects' covariates, and study design as in the analysis data set. In order to adjust for the differences in e.g. actual doses, a pcVPC will be performed in which the observed and simulated DV will be normalized based on the typical population prediction.

8. REFERENCES

1.	*CPMP/ICH/363/96:* "Statistical Principles for Clinical Trials", ICH Guideline Topic E9, Note For Guidance on Statistical Principles for Clinical Trials, current version.
2.	*001-MCS-40-413:* Identify and Manage Important Protocol Deviations (iPD)", current version, Group "Clinical Operations", IDEA for CON.
3.	*001-MCS-40-135_RD-01:* "Integrated Quality and Risk Management Plan", current version, Group "Clinical Operations", IDEA for CON.
4.	*001-MCG-156_RD-01:* "Handling of missing and incomplete AE dates", current version; IDEA for CON.
5.	*001-MCG-159_RD-06*: "Standard table shells for inferential and descriptive Company Standard Displays (CSD-Catalogue)", current version; IDEA for CON.
6.	*001-MCG-159*: "Reporting of Clinical Trials and Project Summaries", current version; IDEA for CON.
7.	*001-MCG-156*: "Handling and summarization of adverse event data for clinical trial reports and integrated summaries", current version; IDEA for CON.
8.	REGULATION (EU) No 536/2014 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 16 April 2014 on clinical trials on medicinal products for human use, and repealing Directive 2001/20/EC, European Commission webpage.
9.	*001-MCG-157*: "Handling, Display and Analysis of Laboratory Data", current version; IDEA for CON.
10.	WHO Working Group on the Clinical Characterisation and Management of COVID-19 infection. A minimal common outcome measure set for COVID-19 clinical research. Lancet Infectious Diseases, Published Online June 12, 2020, doi: 10.1016/S1473-3099(20)30483-7; 2020. [R20-2002]
11.	Schulman S, Kearon C. Subcommittee on Control of Anticoagulation of the Scientific and Standardization Committee of the International Society on Thrombosis and Haemostasis. Definition of major bleeding in clinical investigations of antihemostatic medicinal products in non-surgical patients. J Thromb Haemost. 2005. 3: 692-694. [R05-0344]
12.	Bergstrand M, Hooker AC, Wallin JE, Karlsson MO Prediction-corrected visual predictive checks for diagnosing nonlinear mixed-effects models. AAPS J 2011 ; 13(2) ; 143-151. [R13-0300]



10. HISTORY TABLE

Table 10: 1 History table

Version	Date (DD-MMM-YY)	Author	Sections changed	Brief description of change
1.0	**17-DEC-2020**	[redacted]	None	This is the final TSAP.
2.0	**03-May-2020**		2	Administrative: Added abbreviation for APACHE.
			4	Administrative: Included all changes, including also for TSAP V1.
			6.6	Administrative: Addition of clarification sentence for SOFA.
			7.8.1	Administrative: Remove text on collapsing of AEs to be in line with new template.
			8	Administrative: Reference format same for all.
			7.4.2	Addition of sensitivity analysis: Estimate of unadjusted treatment effect for primary endpoint, main estimand.
			7.5.2	Addition of sensitivity analyses: Estimates of unadjusted treatment effects for MBE, ACM, ACM/MV, VFD at Day 28. Estimate of adjusted treatment effect for PaO2/FiO2 ratio at Day 6.
			4, 5.2.2	Clarification: TEAE text added to the secondary endpoint ‘MBE until Day 6’
			4, 5.3, 7.4.1	Clarification: Patients with WHO=9 at baseline are

				accepted and text updated accordingly.
			4, 6.7	Clarification: Definition of 'on treatment' to start from first administration of alteplase / randomisation for active / SOC groups, and to last for 288h (=12days). For alteplase with >5d then end of 'on treatment' is last infusion + 168h (7d).
			4, 6.4, 7.4.2	Addition of sensitivity analyses, requested by Brazilian authorities: New subgroups for investigation of primary endpoint: 1) Participation in more than 1 stud for COVID, started either prior to or after randomisation 2) Intake of conmeds for COVID-19 (yes/no) 3) Type of heparin intake, either UFH, LMWH, then low / mod / high dose intensity. Patients with comorbidities, e.g. diabetes, overweight, age, cardiac comorbidities.

TRIAL STATISTICAL ANALYSIS PLAN (PART 2 ANALYSIS)

c37258408-01

BI Trial No.:	0135-0347
Title:	The **TRISTARDS** trial - **T**h**R**ombolys**IS** **T**herapy for **ARDS** A Phase IIb/III operationally seamless, open-label, randomised, sequential, parallel-group adaptive study to evaluate the efficacy and safety of daily intravenous alteplase treatment given up to 5 days on top of standard of care (SOC) compared with SOC alone, in patients with acute respiratory distress syndrome (ARDS) triggered by COVID-19.
Protocol Version:	03 (05Oct2021)
Investigational Product(s):	Alteplase (recombinant tissue-type plasminogen activator, rt-PA)
Responsible trial statistician(s):	[redacted] Tel.: [redacted] Fax:
Date of statistical analysis plan:	26 OCT 2021 SIGNED
Version:	1

1. TABLE OF CONTENTS

LIST OF TABLES

2. LIST OF ABBREVIATIONS

Term	Definition / description
ACM	All cause mortality
AE	Adverse events
ALT	alanine transaminase
AME	Average marginal effect
AMI	Acute myocardial infarction
ANCOVA	Analysis of Covariance
aPTT	activated partial thromboplastin time
ARDS	Acute respiratory distress syndrome
AST	aspartate transaminase
AUC	Area under the curve
BRAVE	BI Rave
BMI	Body mass index
CI	Confidence interval
COVID-19	coronavirus disease 2019
CRF	Case report form
CRP	C-reactive protein
CTP	Clinical Trial Protocol
CWRES	Conditional weighted residuals
DVT	Deep Vein Thrombosis
ECMO	Extracorporal membrane oxygenation
eGFR	Estimated glomerular filtration rate
EMA	European Medicines Agency
EU	European Union
EudraCT	European Union Drug Regulating Authorities Clinical Trials Database
FAS	Full Analysis Set
GGT	Gamma-Glutamyl Transferase
HR	Hazard ratio
ICU	Intensive care unit
IMV	Invasive mechanical ventilation
INR	International normalised ratio

Term	Definition / description
iPD	Important protocol deviation
IRT	Interactive response technology
ISTH	International Society on Thrombosis and Haemostasis
LMWH	Low molecular weight heparin
MBE	Major Bleeding Event
MedDRA	Medical Dictionary for Drug Regulatory Activities
MI	Myocardial infarction
MV	Mechanical Ventilation
NIV	Non invasive Ventilation
OFD	Oxygen-free days
PaO_2/FiO_2	Oxidation Index (Horowitz Index)
pcVPC	Predicted corrected visual predictive check
PE	Pulmonary Embolism
PK	Pharmacokinetic
PKS	Pharmacokinetic Set
PPS	Per Protocol Set
PRED	Predictions
RBC	Red blood cells
RD	Risk difference
REML	Restricted maximum likelihood
REP	Residual effect period
RPM	Report planning meeting
RS	Randomised Set
rt-PA	recombinant tissue-type plasminogen activator
SARS-CoV-2	severe acute respiratory syndrome coronavirus 2
SCR	Screening Set
SD	Standard deviation
SE	Standard error
SOC	Standard of Care
SOFA	Sequential (sepsis-related) Organ Failure Assessment
SpO_2	Oxygen saturation
TEAE	Treatment emergent adverse events

Term	Definition / description
TPA	Tissue Plasminogen activator
TS	Treated Set
TSAP	Trial Statistical Analysis Plan
UFH	Unfractionated heparin
ULN	Upper limit of normal
VFD	Ventilator Free Days
WBC	White blood cells
WHO	World Health Organisation

3. INTRODUCTION

As per ICH E9 (1), the purpose of this document is to provide a more technical and detailed elaboration of the principal features of the analysis, as described in the clinical trial protocol (CTP) Version 3 (c32434345-03, 2). It will detail the procedures for executing the statistical analysis of the primary, all secondary endpoints, and other data, and will be limited to the analysis of Part 2 of BI trial 0135-0347.

CTP Version 3 is the updated CTP, following the interim results of Part 1. This TSAP assumes familiarity with CTP Version 3. In particular, the TSAP is based on the planned analysis specifications as written in CTP Version 3 Section 7 “Statistical Methods and Determination of Sample Size”. Therefore, TSAP readers may consult the CTP Version 3 for more background information on the study, e.g., on study objectives, study design and population, treatments, definition of measurements and variables, and planning of sample size and randomisation.

The NIV (non-invasive ventilation) patient cohort are those with a baseline WHO value of 6, and the IMV (invasive mechanical ventilation) patient cohort are those patients with a value of 7, 8 or 9 at baseline. All analyses will be performed within the NIV patient cohort as the main cohort for confirmatory purposes, and separately within the IMV patient cohort for exploratory purposes. This is consistent with the hierarchy testing approach following the primary and key secondary objectives of the statistical analysis, as outlined in CTP version 3 Section 7. Select analyses will be performed on the NIV and IMV cohorts pooled and the extent of the analyses will depend on the homogeneity observed of the two groups.

A database snapshot will be made when all patients have completed the day 28 visit and when the data has been cleaned. At this point, all analyses outlined in this Trial Statistical Analysis Plan (TSAP) will be performed, except for all cause mortality (ACM) at day 90. This further endpoint will be available at a later timepoint. When this data is available, then another snapshot will be taken, the database will be locked, and this endpoint will be analysed.

This is an open-label study, therefore no blinding will apply for patients, investigators and personnel at the site involved in trial conduct. The access to the randomisation code list will be kept restricted until it is released for analysis. While the study is in progress, access to tabular results of study outcomes by treatment allocation will not be made available to the patients, investigators and personnel at the site, to the trial statistician, clinical team, or members of the steering committee (unless the DMC advises otherwise). The DMC and DMC statisticians will be unblinded.

A separate abbreviated TSAP will be written to cover pooled analyses of Part 1 and Part 2 for SOC alone and the common alteplase group. Within that abbreviated TSAP, efficacy analyses will be limited to primary and secondary efficacy endpoints (as defined in Part 2) and to the NIV patient cohort.

The trial data is stored in the BI Rave (BRAVE) database system. SAS® Version 9.4 or later will be used for all analyses, except for population PK as outlined in Section 7.8.5.1.

4. CHANGES IN THE PLANNED ANALYSIS OF THE STUDY

The screened set (SCR) and pharmacokinetic set (PKS), not outlined in CTP Version 3, are now defined in Section 6.3. They are needed to summarise those patients to be included in the disposition flow and the PK analysis.

Subgroup analyses not included in CTP Version 3 are now outlined in Sections 6.4, 7.4.2 and 7.5.2 for the primary endpoint and one of the secondary endpoints. These analyses are to be used to test the robustness of the main analyses for that particular endpoint and to investigate if any of the subgroups are having an overwhelming influence on the study results.

5. ENDPOINTS

5.1 PRIMARY ENDPOINT

The primary endpoint as defined in CTP Version 3 Section 2.1.2 will be used and will be analysed on the Full analysis set (FAS). This is based on the daily assessment of the WHO Clinical Progression scale (3), which ranges from 0 to 10. Clearly, a shorter time to clinical improvement or hospital discharge reflects a better outcome.

> "Time to clinical improvement or hospital discharge up to day 28, defined as the time from randomisation to either an improvement of two points on the 11-point WHO Clinical Progression Scale or discharge from the hospital, whichever comes first."

However, there are several elements which have to be taken into account, when deriving the endpoint, and in order to address the hypothetical estimand. These are already specified in the CTP Version 3 and summarized in Table 5.1: 1 below.

In addition, in the unlikely event that a patient discontinues from the study due to withdrawal of consent, prior to being able to determine if he had clinical improvement or hospital discharge, or had intake of bail-out therapy or death, he will be censored at the time of study withdrawal. This has not been included in the protocol definition but is considered only as a clarification.

Bail-out therapy is as defined in CTP Version 3 Section 4.2.2.1 Ultima ratio situation - Bail-out, which is 'marketed Actilyse®'. The various scenarios for handling of intake of bail-out therapy are summarized in Table 5.1: 1 below:

Table 5.1: 1 Censoring of the primary endpoint for the main estimand

Scenarios	**Main Estimand: Hypothetical situation**
Bail-out then death	Censor at Bail-out
Bail-out then failure to improve#	Censor at Bail-out
Bail-out then clinical improvement / hospital discharge	Censor at Bail-out
Bail-out then clinical improvement / hospital discharge then death	Censor at Bail-out
Clinical improvement / hospital discharge regardless of subsequent events	Positive event at clinical improvement / hospital discharge
Death only, prior to day 28	Censor at day 28
Failure to improve# by day 28	Censor at day 28
Discontinued from study due to withdrawal of consent prior to clinical improvement / hospital discharge or Bail-out/Death	Censor at time of withdrawal

Clinical improvement / hospital discharge refers to the first of clinical improvement or hospital discharge, whichever comes first.
#Failure to improve is failure to have clinical improvement or hospital discharge.

The planned analyses of the primary endpoint are outlined in Section 7.4.1, and the supplementary and sensitivity analyses of this endpoint in Section 7.4.2.

5.2 SECONDARY ENDPOINTS

5.2.1 Key secondary endpoints

The two key secondary endpoints as defined in CTP Version 3 Section 2.1.3 will be analysed on the FAS, and these are both based on the daily assessment of the WHO Clinical Progression scale.

The planned analyses of the key secondary endpoints are outlined in Section 7.5.1.

Treatment failure (ACM / MV) at day 28, FAS, binary endpoint
This unfavorable endpoint is met if:

- the last known status of the patient has a value of ≥7 on the WHO clinical progression scale, up to end of day 28, or
- vital status is dead within 28 days

ACM at day 28, FAS, binary endpoint
This unfavorable endpoint is met if:

- the last known status of the patient is 10 on the WHO clinical progression scale by the end of day 28, or
- vital status is dead within 28 days

5.2.2 Other secondary endpoints

The secondary endpoints as defined in CTP Version 3 Section 2.1.3 which are not listed under "Key secondary endpoints" will be referred to as "Other secondary endpoints". Efficacy endpoints will be analysed on the FAS, and the one safety endpoint "Major bleeding event (MBE) until day 6" will be analysed on the Treated Set (TS).

The planned analyses of the secondary endpoints are outlined in Section 7.5.2.

MBE (according to International Society on Thrombosis and Haemostasis [International Society on Thrombosis and Haemostasis (ISTH) definition (4)]) until day 6, TS, binary endpoint
If the patient has an MBE, at any timepoint up to end of day 6 then he has met this unfavorable endpoint. If it is unknown whether the patient had an MBE up to end of day 6, regardless of the reason, then it will be assumed that the patient did not have an MBE by the end of day 6. This is a safety defined endpoint and the starting point for consideration should be consistent with the 'on treatment' definition in Section 6.7. The time period for counting events is defined in Table 5.2.2: 1 below:

Table 5.2.2: 1 Time period for counting MBE up to day 6

MBE until day 6	Start point	End date
SOC patients	Randomisation date/time	Start point + 144 hrs
Alteplase patients	First administration of alteplase date/time	Latest time of: ○ Start point + 144 hrs ○ Last administration of alteplase date/time + 24 hrs

[Note: 144 hrs represents 6 days]

PaO_2/FiO_2 ratio (or inferred PaO_2/FiO_2 ratio from SpO_2) change from baseline to day 6, FAS, continuous endpoint
This assessment is planned to be measured on each of days 0 to 7, and again on day 28, but only whilst the patients is still in hospital. For this endpoint, the worst (lowest) daily measurement will be used and the higher the value the better the health status of the patient.

- If the patient is still in hospital during day 6 then the day 6 value will be used
- If the patient has been discharged from hospital prior to day 6 then the value at the time of hospital discharge will be used
- If the patient has died prior to day 6 then the last value prior to death will be used
- If day 6 value is missing but Day 5 value available, the day 5 value will be used

- If day 6 value is missing, no Day 5 value available, but day 7 available, then day 7 value will be used
- Otherwise value set to missing for that patient.

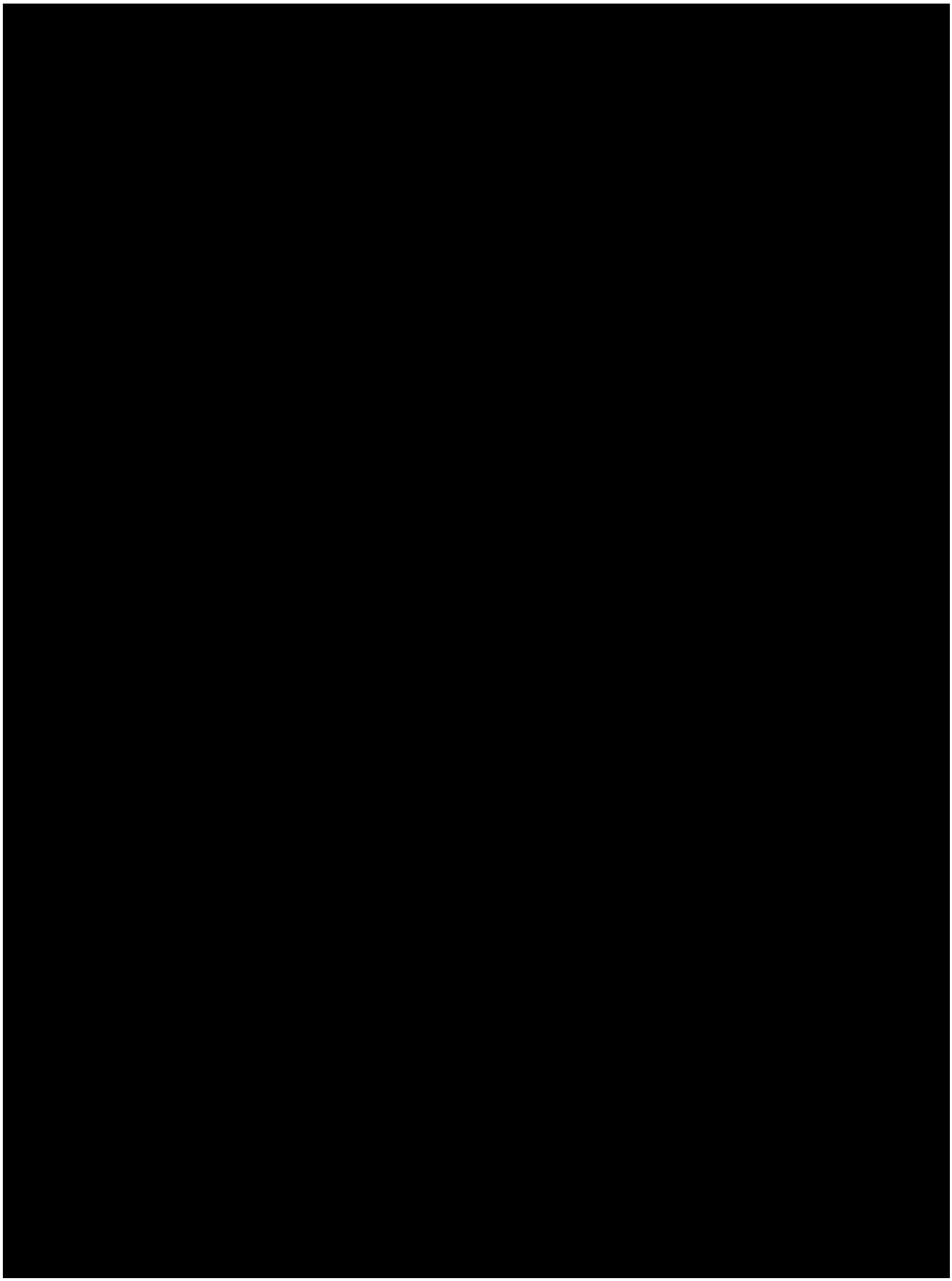
Based upon this, the change from baseline for each patient will be calculated and used for the analysis.

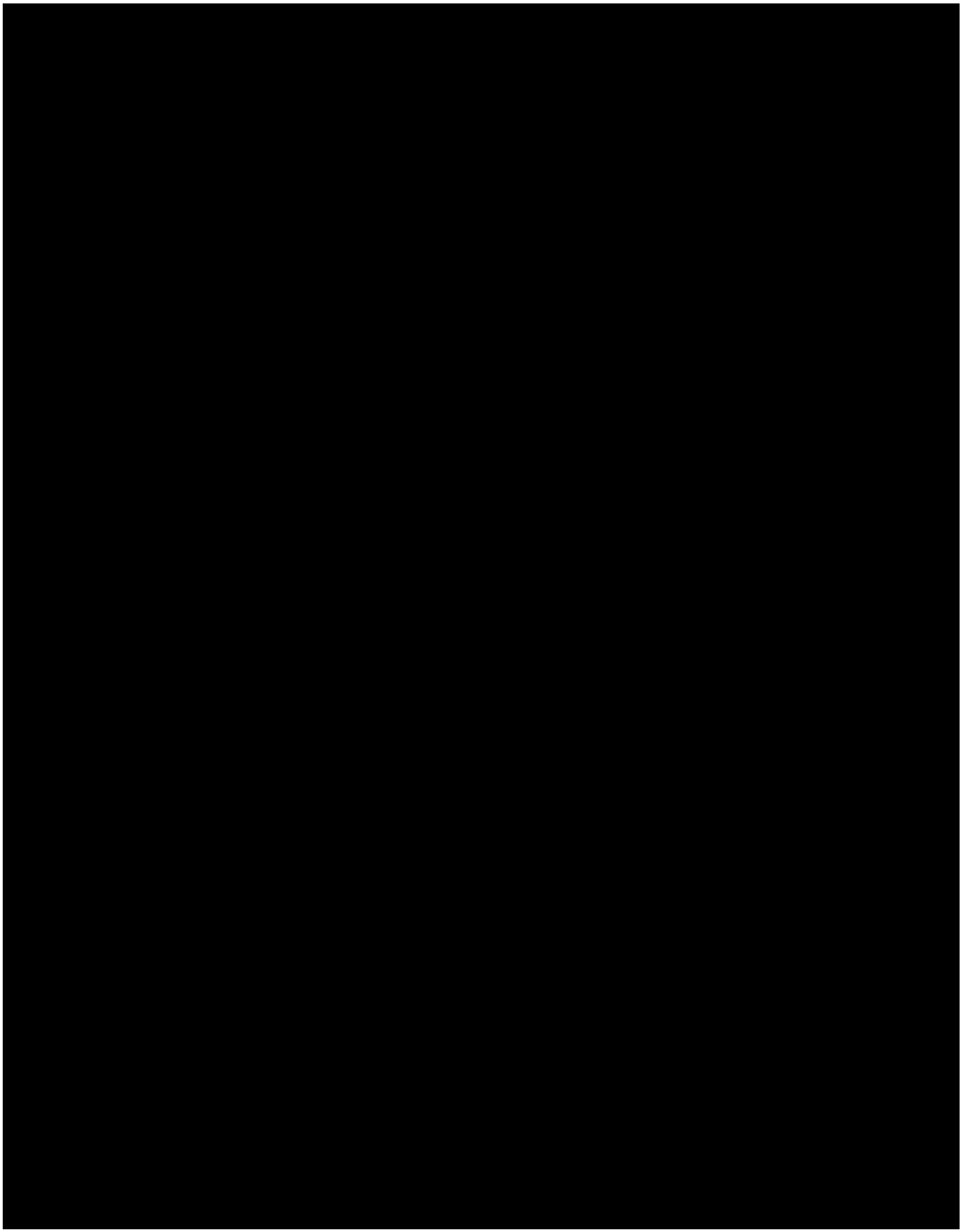
Number of oxygen-free days (OFD) up to day 28, FAS, continuous endpoint

For this endpoint, 'oxygen-free' is defined as free from assistance from oxygen support. The number of oxygen-free days starts from when the patient has a 'lasting' value on the WHO clinical progression scale of ≤ 4 and ends on Day 28. A lasting value of ≤ 4 means that the value cannot exceed 4 at a later timepoint. If the patient is liberated from oxygen on Day x, then the number of OFDs is 28-x. If a patient has withdrawn consent prior to day 28 then he will have a missing value for OFD. In any case, if the status of the patient at Day 28 is death, as determined from the vital status page then the OFD=0.

Length of hospital stay up to day 28, FAS, continuous endpoint

This will be determined based upon the first hospital discharge date, or discharge to another care facility. If the patient dies within the first 28 day period, then length of hospital stay is 28.





6. GENERAL ANALYSIS DEFINITIONS

6.1 TREATMENTS

For the purposes of the statistical analyses, treatments will be referred to in the same context as in CTP Version 3 Section 7.4.

- "Alteplase", which is alteplase 0.6 mg/kg over 2 hours followed by 0.04 mg/kg/h over 12 hours

- Standard of Care (SOC)

Alteplase treatment is planned to be up to end of Day 5 but all patients should be taking SOC in the background. On day 1 there is an initial 2 h infusion for all, followed up by a longterm 12 h infusion. On days 2-5 the patients can receive an optional 2h infusion (based on investigator decision), but only on one of these days. Each patient should receive a longterm 12h infusion on these remaining 4 days. Some patients may have a temporary interruption, as advised by CTP Version 3 Section 3.3.4.1.

6.2 IMPORTANT PROTOCOL DEVIATIONS

Patients with any of the following important protocol deviations (iPDs) will be reviewed on a case by case basis (5) and (6). The review of the iPDs will be used to determine criteria for a PPS.

Note that this is a working list and may be updated again prior to the final Report Planning Meeting (RPM) prior to the snapshot related to end of Part 2. Inclusion criteria/exclusion criteria numbers refer to the definition given in the CTP Version 3 or CRF.

Table 6.2: 1 Handling of iPDs

iPD code	**iPD Category & Brief Description**
A	**Entrance Criteria Not Met**
A1	**Inclusion Criteria not met**
A1.1	Patient too young (IC1)
A1.2	Diagnosis of ARDS questionable (IC2,4,5)
A1.3	Patient does not test positive for SARS-CoV-2 (IC3)
A2	**Exclusion Criteria not met**
A2.1	Patient has baseline conditions that are not permitted for safety reasons (EC1-33)

Table 6.2: 1 Handling of iPDs (continued)

iPD code	iPD Category & Brief Description
A2.2	Patient has baseline conditions that prevent patient from complying with study protocol (EC1-33)
A2.3	Patient has had previous conditions or procedures that are not permitted (EC1-33)
A2.4	Patient has had forbidden previous therapy (EC1-33)
A2.5	Patient pregnant (EC27)
B	**Informed Consent**
B1	Informed consent not available (IC6)
B2	Informed consent not signed at screening visit 1 (IC6)
B3	Informed consent not available or not available at visit 2a (pre-dose) (IC6)
C	**Trial medication and randomisation**
C1	Method of assigning patients to treatment arms
C1.1	Randomisation not followed according to protocol
C1.2	Patients do not receive the initial treatment they were randomised / allocated to
C1.3	Patient assignment not followed
C1.4	Timeframe > 1 day between randomisation and first drug intake
C2	**Non-compliance**
C2.1	Overall Compliance not between 80% and 120% inclusive – see definition in Section 5.4.
C2.2	Patient takes the optional 2h infusion on more than one occasion after the initial 2h infusion.
C3	**Drug assignment and administration of doses for each patient**
C3.1	Dosage and treatment schedule not given according to protocol for initial iv infusion
C3.2	Dosage and treatment schedule not given according to protocol for long term iv infusion
C3.3	Drug not permanently discontinued according to CTP Version 3 Section 3.3.4.1
C3.4	Drug not temporarily interrupted according to CTP Version 3 Section 3.3.4.1

Table 6.2: 1 Handling of iPDs (continued)

iPD code	iPD Category & Brief Description
D	**Concomitant medication**
D1	Intake of restricted concomitant medication according to CTP Version 3 Section 4.2.2.1 and Section 10.2
D2	Intake of marketed Actilyse® (non-study drug) not as ultima-ratio or bail-out
G	**Other trial specific important deviations**
G1	Other protocol violations affecting patient rights or safety (manual PDs to be captured)

6.3 SUBJECT SETS ANALYSED

The subject sets as defined in CTP Version 3 Section 7 for the Randomised Set (RS), Full Analysis Set (FAS) and Treated Set (TS) will be used.

The Screened set (SCR), not outlined in the CTP Version 3, will include all patients screened.

The RS will consist of all randomised patients.

The FAS will consist of all randomised patients with at least one baseline and one post baseline assessment relating to the primary endpoint of interest. That is to say, they need to have at least one assessment post baseline on the 11-point WHO Clinical Progression Scale.

The TS will consist of all patients who were randomised and, for patients in the alteplase group, treated with at least one dose of trial drug.

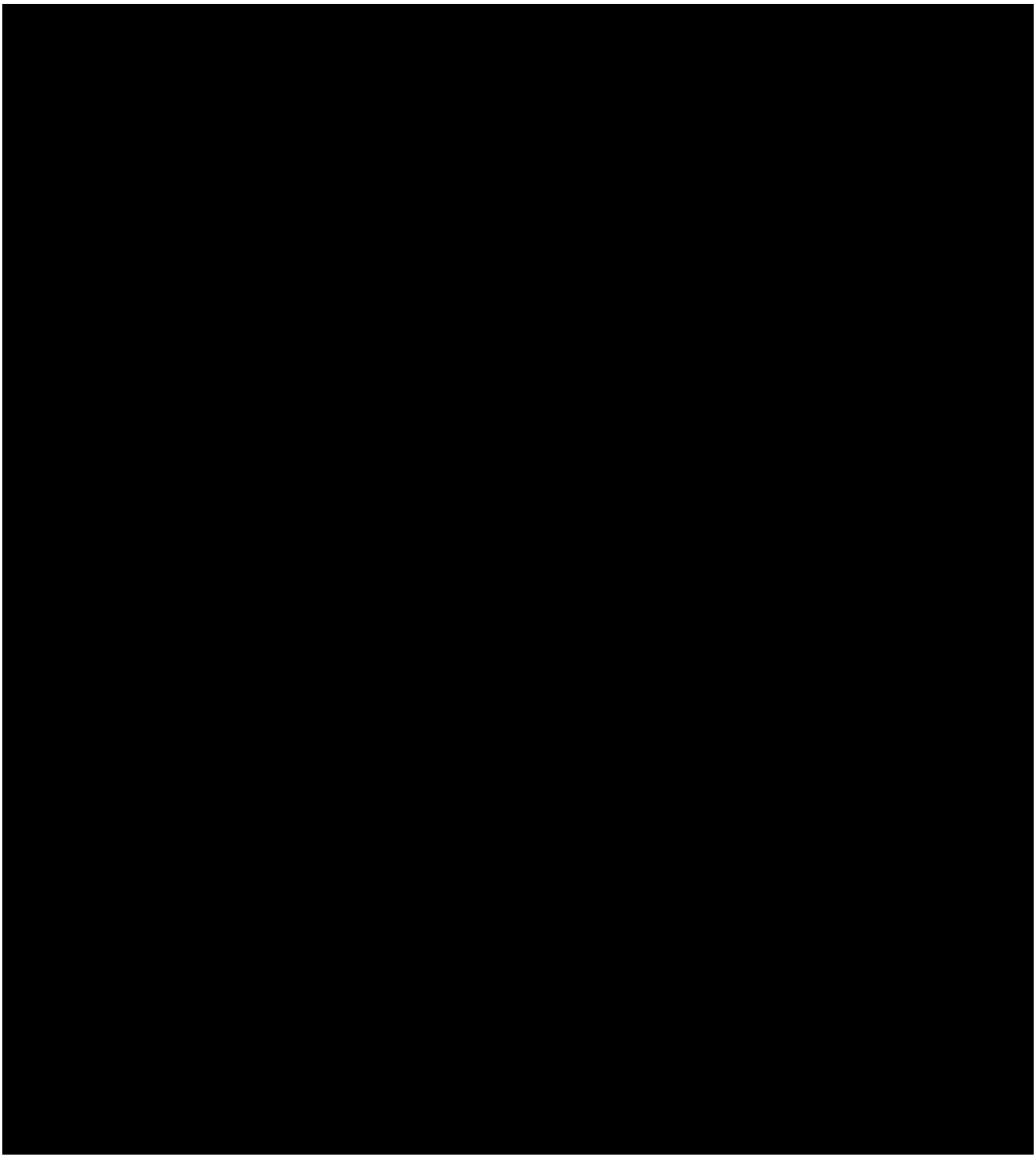
Per Protocol Set (PPS) will consist of all patients in FAS who were without important protocol deviations (IPDs) for efficacy, as outlined in Table 6.2: 1.

The Pharmacokinetic set (PKS), not outlined in CTP Version 3, will comprise all subjects in the TS who provided at least one PK endpoint and had no important protocol violations relevant to the evaluation of PK.

Table 6.3: 1 below outlines which analysis set is to be used for the various endpoints or summaries.

Table 6.3: 1 Subject sets analysed

Class of endpoint	Subject set				
	SCR / RS	**TS**	**FAS**	**PPS**	**PKS**
Primary endpoint			primary and supplementary analyses	Primary analyses	
Key secondary endpoints			x	Primary analyses	
Other secondary EFFICACY and further EFFICACY endpoints			X		
Secondary SAFETY and further SAFETY endpoints		X			
Treatment exposure		X			
Disposition	X				
Demographic/baseline		X	X	x	
Concomitant medications and compliance		X	X		
All other safety analyses		X			
PK analyses					X



6.5 POOLING OF CENTRES

Part 2 of this study is planned to be carried out in approximately 110-130 centres in approximately 22 countries. However, not all centres have the same SOC procedures. During the course of the study, and gathering information (retrospectively) from each of the centres

with regard to their SOC procedures, they will be grouped into relatively homogeneous groupings. The same process as done for Part 1 will be carried out again for Part 2.

Because the groupings will only be made after randomisation of a patient within a centre, this will be a retrospective and exploratory analysis, albeit planned. It is planned only to investigate the primary endpoint with this centre grouping in a sensitivity analysis – see Section 7.4.2.

6.6 HANDLING OF MISSING DATA AND OUTLIERS

All efforts will be made to avoid having missing data in the database, however it is inevitable that there will be missing data, by design:

- For patients that die, there will be no further data, however this irreversible unfavourable state will be taken into consideration in the determination of each of the endpoints.
- For patients that are discharged from hospital before day 28, the only additional efficacy assessment is the vital status at days 28 and 90. The fact that the patient has improved in order to be discharged from hospital prior to day 28 will be taken into account in the determination of each of the endpoints.

Handling of missing data for the endpoints are outlined in Section 5, in line with the definitions of the endpoints.

Missing or incomplete adverse event (AE) dates will be computed using the BI handling rules (7).

6.7 BASELINE, TIME WINDOWS AND CALCULATED VISITS

Randomisation is defined as Day 1 and it is planned that the alteplase patients are administered their initial 2h infusion of study medication within 6 hours of randomisation, on Day 1 (Visit 2a). The last planned administration of study medication for the alteplase patients is the long term 12h infusion on Day 5.

Since all patients are on SOC and there is no clear start or stop of SOC, a fixed treatment duration will be used for all patients, for the purposes of the analyses. Therefore, endpoints referred to as ‘on treatment’ will include all relevant events from the relevant start point plus 288 hours (last planned treatment on Day 5 + 7 days of REP = 12 days = 288 hrs).

However, to take account of the fact that some patients may have had treatment interruptions, the residual effect period should be in relation to the last intake of alteplase infusion. Table 6.7: 1 below outlines the time period definitions for counting on treatment safety event.

Table 6.7: 1 Time period for counting on treatment safety events

On treatment	Start point	End date
SOC patients	Randomisation date/time	Start point + 288 hrs
Alteplase patients	First administration of alteplase date/time	Latest of: o Start point + 288 hrs o Last administration of alteplase date/time + 168 hrs

[Note: 288 hrs represents 12 days, 168 hrs represents 7 days]

According to CTP Version 3 Section 1.2, the residual effect period (REP) is 7 days.

As outlined in CTP Version 3 Section 7.2.1, the term "baseline" refers to the last observed measurement prior to randomisation on Day 1.

7. PLANNED ANALYSIS

The tables that are created from the statistical analysis will follow the format of the standard within BI (8).

The overall disposition of the patients in the trial will be presented, together with the number of patients discontinuing prior to day 28, and vital status at day 90.

The number of patients participating in the study by country and the number of sites, for each of the patient populations will be summarised.

Demography and baseline characteristics will be presented, as well as the stratification used at the time of the patient randomisation. A summary of the iPDs will be provided.

Where statistical comparisons are indicated, they will be done by comparing alteplase versus SOC.

All descriptive analyses will be performed:
- within the NIV patient cohort as the main cohort
- within the IMV patient cohort as the exploratory cohort
- on the NIV and IMV cohorts pooled – additional select efficacy analyses for primary and secondary efficacy endpoints

For the NIV patient cohort, the default set of covariates for adjustment in analyses are:
- Baseline D-Dimer status (≥3 to <5, ≥5-fold ULN)
- Age
- Number of days of NIV support
- Baseline value of the PaO_2/FiO_2 ratio – only for the endpoint PaO_2/FiO_2 ratio change from baseline to Day 6

For the IMV patient cohort, the default set of covariates for adjustment in analyses are:
- Baseline D-Dimer status (≥3 to <5, ≥5-fold ULN)
- Age
- Baseline WHO value (7, 8, 9)
- Baseline value of the PaO_2/FiO_2 ratio – only for the endpoint PaO_2/FiO_2 ratio change from baseline to Day 6

For efficacy analyses, patients will be analysed according to the patient information given in the CRF, in the case of potentially erroneous data entered into the interactive response technology (IRT). This is related to the baseline information, ventilaton support (NIV, IMV) and D-Dimer level (≥3 to <5, ≥5-fold ULN).

In general, for End-Of-Text tables, the set of summary statistics is: N (number of patients with non-missing values) / Mean / SD / standard error (SE) / Min / Q1 (lower quartile)/ Median / Q3 (upper quartile)/ Max.

Statistical parameters will be displayed to a defined number of decimal places as specified in the BI guideline "Reporting of Clinical Trials and Project Summaries" (9).

Tabulations of frequencies for categorical data will include all possible categories and will display the number of observations in a category as well as the percentage (%) relative to the respective treatment group (unless otherwise specified, all patients in the respective patient set whether they have non-missing values or not). Percentages will be rounded to one decimal place. The category missing will be displayed only if there are actually missing values.

7.1 DEMOGRAPHIC AND OTHER BASELINE CHARACTERISTICS

Only descriptive statistics are planned for this section of the report. See Section 5.4 for specific definitions.

For the two randomisation stratification factors at baseline, baseline ventilation support (NIV, IMV) and baseline D-Dimer level (≥3 to <5, ≥5-fold ULN), any discrepancies between the IRT and the CRF will be documented and summarized. Should there be a discrepancy between the value used for the randomisation stratification and the actual value as determined on the CRF, the actual value as determined on the CRF will be used in the analyses.

Within this section, all analyses will be performed on the NIV patient cohort, the IMV patient cohort and on the NIV and IMV cohorts pooled.

7.2 CONCOMITANT DISEASES AND MEDICATION

Medical history, COVID-19 signs and symptoms and concomitant diagnoses will be presented and coded using MeDRA System Organ Class and Preferred Term where possible.

Regarding concomitant medications, there is a specific question asking if the treatment was given as part of standard of care for the treatment of COVID-19, which is relevant for all patients since they all receive SOC in the background. This question is different to the randomised treatment assignment. Therefore, all concomitant medication analyses or displays will be split by 'Standard of Care for the treatment of COVID-19', yes or no. All concomitant medications will be coded using the WHO Drug dictionary.

Medications of special interest for COVID (as indicated by WHO, see Section 10.1), and restricted medications within this study (as outlined in CTP Version 3 Section 4.2.2.1, see Section 10.2) will also be summarized and split by whether or not the medication was given as part of the Standard of Care for the treatment of COVID-19.

Use of heparin will be summarized and presented.

Therefore, only descriptive statistics are planned for this section of the report.

Within this section, all analyses will be performed on the NIV patient cohort, the IMV patient cohort and on the NIV and IMV cohorts pooled.

7.3 TREATMENT COMPLIANCE

Only descriptive statistics are planned for this section of the report, and due to the nature of the study, compliance will be determined for the alteplase treatment group only.

Compliance to intake of infusion will be assessed in terms of % taken, as measured by volume, at each planned infusion. Overall compliance will be as an average measure of the %taken. The detailed definitions are provided in Section 5.4.

Compliance with preparation of the infusion in terms of concentration, as indicated in CTP Version 3 Table 10.4 are also assessed, and detailed definitions provided in Section 5.4.

Within this section, all analyses will be performed on the NIV patient cohort, the IMV patient cohort and on the NIV and IMV cohorts pooled.

7.4 PRIMARY ENDPOINT

Within this section, all descriptive analyses will be performed on the NIV patient cohort, the IMV patient cohort and on the NIV and IMV cohorts pooled.

7.4.1 Primary analysis of the primary endpoint

For the primary endpoint, a Cox proportional hazards model will be used to estimate the hazard ratio (HR) comparing alteplase to SOC. From the model, the 95% confidence intervals (CI) and corresponding Wald p-values for the HRs will be produced. The model will include fixed effects for treatment (alteplase, SOC), and the default set of covariates for the respective NIV or IMV patient cohorts, as outlined in Section 7.

Kaplan Meier estimates will be presented by treatment (alteplase, SOC) for this endpoint up to day 28, and additionally, for the NIV patient cohort:

- treatment and baseline D-Dimer status (≥3 to <5, ≥5-fold ULN)
- treatment and age (≤median, >median)
- treatment and number of days of NIV support (≤median, >median)

and for the IMV patient cohort:

- treatment and baseline D-Dimer status (≥3 to <5, ≥5-fold ULN)
- treatment and age (≤median, >median)
- treatment and baseline WHO status (7, 8, 9)

From the KM estimates at various timepoints (days 6, 8, 12, 16, 20, 24 and 28) the risk difference (RD) for the treatments with 95% CI will be estimated by taking the difference of the individual probabilities. Since the observations in the two treatment groups are

independent, the variance of the Kaplan-Meier probability difference is equal to the sum of the Kaplan Meier probabilities' variances found by Greenwood's method. The 95% CI of the RD is therefore calculated accordingly.

7.4.2 Sensitivity analysis, subgroup analysis, exploratory analysis of the primary endpoint

Two non-confirmatory supplementary analyses will be conducted, in anticipation of the rare situation where bail-out therapy may be used (as defined in CTP Version 3 Section 4.2.2). They will be performed on the FAS.

The first of these supplementary analyses will address the treatment policy estimand, whereby there is no consideration for the intake of bail-out therapy.

The second of these supplementary analyses will address a composite estimand, whereby patients receiving bail-out therapy will be censored at day 28, rather than on the day of bail-out. Here, bail-out represents treatment failure and thereby, by definition, precludes any response after bail-out. The various scenarios are summarized in Table 7.4.2: 1 below.

In both cases, and in the unlikely event that a patient discontinues from the study, prior to being able to determine if he had clinical improvement or hospital discharge, or had intake of bail-out therapy or death, he will be censored at the time of withdrawal.

Table 7.4.2: 1 Censoring of the primary endpoint for the treatment policy and composite estimands

Scenarios	**Treatment policy estimand**	**Composite estimand**
Bail-out then death	Censor at day 28	Censor at day 28
Bail-out then failure to improve#	Censor at day 28	Censor at day 28
Bail-out then clinical improvement / hospital discharge	Positive event at clinical improvement / hospital discharge	Censor at day 28
Bail-out then clinical improvement / hospital discharge then death	Positive event at clinical improvement / hospital discharge	Censor at day 28
Clinical improvement / hospital discharge regardless of subsequent events	Positive event at clinical improvement / hospital discharge	Positive event at clinical improvement / hospital discharge
Death only, prior to day 28	Censor at day 28	Censor at day 28
Failure to improve# by day 28	Censor at day 28	Censor at day 28
Discontinued from study due to withdrawal of consent prior to clinical improvement / hospital discharge or Bail-out/Death	Censor at time of study discontinuation	Censor at time of withdrawal

Clinical improvement / hospital discharge refers to the first of clinical improvement or hospital discharge, whichever comes first.
#Failure to improve is failure to have clinical improvement or hospital discharge.

The analyses performed will be identical to that outlined for the primary endpoint in Section 7.4.1.

Based on the main estimand, the treatment effect will be estimated using the Cox proportional hazard model, containing only treatment in the model. The HR (95%CI) and p-value estimating the unadjusted treatment effect will be produced.

The effect of the subgroups in the NIV patient cohort, as outlined in Section 6.4, related to the primary endpoint, main estimand, will be assessed descriptively and using Kaplan Meier estimates. Using the Cox proportional hazard model, hazard ratio estimates with 95% CI will be estimated from the model in two different methods:

- Firstly, in a model containing treatment, subgroup and the treatment * subgroup interaction.
- Secondly, in the model containing the default set of covariates as outlined in Section 7.4.1 with this additional covariate added.

A covariate adjusted sensitivity analysis of the primary endpoint, main estimand, will be conducted, to determine the effect of the grouping of centres according to similar types of Standard of care. This will be the same analysis.

For the NIV and IMV cohorts pooled, the Cox proportional hazard model will be used, including the fixed effects for treatment (alteplase, SOC), baseline D-Dimer status (≥3 to <5, ≥5-fold ULN), age and baseline ventilation status (NIV, IMV). From this model, the HR and 95% CI will be produced. In addition to this model, the interaction term for the baseline ventilation status and treatment will be included, and the p-value for this additional term will be used as a test of homogeneity. If the p-value is >0.05 the it can be considered that the two groups are homogeneous, with respect to this primary endpoint.

7.5 SECONDARY ENDPOINTS

Within this section, all descriptive analyses will be performed on the NIV patient cohort, the IMV patient cohort and on the NIV and IMV cohorts pooled.

7.5.1 Key secondary endpoints

The two key secondary efficacy endpoints, outlined in Section 5.2.1, are binary in nature and will be analysed on the FAS for both the NIV patient cohort and the IMV patient cohort.

7.5.1.1 Primary analysis of the key secondary endpoints

The risk difference (RD) will be estimated between the two treatment arms using logistic regression. In addition to treatment, adjustment will be made for the default set of covariates, according to the NIV or IMV patient cohort as outlined in Section 7. RD and CIs (95%) for the RD will be obtained using the Delta method and the average marginal effect (AME) method.

7.5.1.2 Sensitivity analysis, subgroup analysis, exploratory analysis of the key secondary endpoints

As sensitivity analysis for each of these binary efficacy endpoints, the unadjusted treatment estimates will be produced, using the same logistic regression model containing only treatment.

For the NIV and IMV cohorts pooled, the RD and 95% CI will be estimated between the two treatment arms using logistic regression, the Delta and the average marginal effect (AME) method. Adjustment will be made for treatment (alteplase, SOC), baseline D-Dimer status (≥3 to <5, ≥5-fold ULN), age and baseline ventilation status (NIV, IMV). In addition to this model, the interaction term for the baseline ventilation status and treatment will be included, and the p-value for this additional term will be used as a test of homogeneity. If the p-value is >0.05 the it can be considered that the two groups are homogeneous, with respect to these two key secondary efficacy endpoints.

7.5.2 Other Secondary endpoints

Other secondary endpoints are defined in Section 5.2.2. In all cases, standard descriptive statistics will be presented, whether the endpoint is binary or continuous.

Binary safety endpoint
For the binary safety endpoint MBE up to day 6, analysed on the TS, there will be no adjustment for covariates. Therefore, the RD will be the difference between the two incidences and the p-value and 95% CI will be determined using the Chan and Zhang exact method.

For the NIV and IMV cohorts pooled, the RD 95% CI will be estimated between the two treatment arms using the same approach as for the key secondary efficacy endpoints. However, for this pooled investigation of MBE up to day 6, the baseline D-Dimer status (≥3 to <5, ≥5-fold ULN) and age covariates will not be included in the model. The p-value for homogeneity will determined similarly.

Continuous efficacy endpoints
The three other secondary endpoints which are continuous in nature will be analysed using a restricted maximum likelihood (REML) based ANCOVA comparing the outcome, by treatment. The ANCOVA model will be adjusted for the default set of covariates as outlined in Section 7 for the respective NIV and IMV patient cohorts. The treatment difference with 95% CI and p-values will be presented.

For the continuous efficacy endpoints, number of OFDs at day 28, and number of days in hospital, there are not expected to be any missing values by definition and as outlined in Section 5.2.2.

For the continuous efficacy endpoint change from baseline in the PaO_2/FiO_2 at day 6, the baseline PaO_2/FiO_2 will also be included in the model as covariate for adjustment. For patients that have died, or who have been discharge from hospital, the handling rules are as outlined in Section 5.2.2. Note, there are expected to be very few deaths or hospital discharges prior to day 6.

In addition, subgroup analyses of this PaO_2/FiO_2 endpoint will be conducted in the NIV patient cohort only and using the subgroups as outlined in Section 6.4. An ANCOVA model will contain the baseline PaO_2/FiO_2, the subgroup of interest and the interaction between subgroup and treatment.

As sensitivity analyses, the unadjusted treatment effects will be estimated, using the same approach as the main analysis, but with only treatment in the model.

Within this section, all descriptive analyses will be performed on the NIV patient cohort, the IMV patient cohort and on the NIV and IMV cohorts pooled.

For the NIV and IMV cohorts pooled, the treatment difference and 95% CI will be estimated between the two treatment arms using a restricted maximum likelihood (REML) based ANCOVA comparing the outcome, by treatment. The ANCOVA model will be adjusted for treatment (alteplase, SOC), baseline D-Dimer status (≥3 to <5, ≥5-fold ULN), age and baseline ventilation status (NIV, IMV). In addition to this model, the interaction term for the baseline ventilation status and treatment will be included, and the p-value for this additional term will be used as a test of homogeneity. If the p-value is >0.05 the it can be considered that the two groups are homogeneous, with respect to these three other secondary efficacy endpoints.

[redacted]

7.7 EXTENT OF EXPOSURE

Exposure will be handled descriptively.

The number of patients with initial 2h infusion on day 1, optional 2h between days 2 and 5, and longterm 12h infusion over the five days of treatment will be summarized, as well as the rate of infusion (mL/kg/h) and duration of infusion (h).

The cumulative exposure will be presented, in terms of Total number of days of infusion. Exposure is relevant for the alteplase treatment groups only.

Within this section, all analyses will be performed on the NIV patient cohort, the IMV patient cohort and on the NIV and IMV cohorts pooled.

7.8 SAFETY ANALYSIS

The safety analysis will be performed as outlined in the CTP Version 3, according to BI standards and on the TS. For further details please see guideline 'Handling and summarization of AE data for clinical trial reports and integrated summaries' (10).

The definition of 'on treatment' is outlined in Section 6.7. Any potential events starting between the time of randomisation and the start of alteplase treatment, will be indicated as such in the listings.

In addition, to fulfil the requirements of the European Union (EU) Regulation 536/2014 (Annex V, Point 6) (11), the following information should be made available, for the lay summary or CTR:

- frequency of serious drug-related AEs by treatment, primary system organ class and preferred term.

Within this section, all analyses will be performed on the NIV patient cohort, the IMV patient cohort and on the NIV and IMV cohorts pooled.

7.8.1 Adverse Events

Unless otherwise specified, the analyses of AE will be descriptive in nature. All analyses of AEs will be based on the number of subjects with AEs and NOT on the number of AEs.

AEs will be coded using the most recent version of the Medical Dictionary for Regulatory Activities (MedDRA) coding dictionary

The analysis of AEs will be based on the concept of treatment emergent adverse events (TEAE). That means that all adverse events occurring 'on treatment' as outlined in Section 6.7 will be considered as TEAE.

All AEs occurring before 'on treatment' will be assigned to 'screening' and all AEs occurring after 'on treatment' will be assigned to 'post-treatment'. In general, AEs attributed to 'Screening' or 'Post-treatment' will be listed only.

An overall summary of AEs will be presented.

Frequencies [N (%)] of patients with AEs will be summarised by treatment, primary system organ class and preferred term (using MedDRA). Separate tables will be provided for patients with serious adverse events.

The system organ classes will be sorted according to the standard sort order specified by European medicines agency (EMA), preferred terms will be sorted by frequency (within system organ class).

Additionally, the following analyses will be reported for disclosure on European Union Drug Regulating Authorities Clinical Trials Database (EudraCT) and clinicaltrials.gov:

- Frequency [N(%)] of patients with non-serious AEs occurring with incidence in preferred term greater than 5% by treatment,
- AEs per arm for disclosure on EudraCT by treatment
- Non-serious AEs for disclosure on EudraCT by treatment
- Serious AEs for disclosure on EudraCT by treatment

Adverse events of interest
All endpoints related to safety, outlined in Section 5.2.2 and Section 5.3, are clearly marked as such, in particular, endpoints related to MBE, Stroke, MI, PE and DVT. There is no further description below on how they should be analysed, however, they will be summarized with the safety data under the topic of 'AEs of special interest'.

There are two other AEs of special interest, which are not mentioned in Sections 5.2.2 and 5.3:

- Transient ischemic attack
- Systemic embolism

AEs recorded on the AE page of the CRF, indicated as such via the relevant tick box, will be grouped and summarized using frequency tables.

7.8.2 Laboratory data

Changes in coagulation and inflammatory markers on treatment, as outlined in Section 5.3, are the laboratory parameters of special interest in this trial. They will be analysed in the efficacy section.

The analyses of laboratory data will be descriptive in nature and will be based on BI standards "Handling, Display and Analysis of Laboratory Data" (12).

The following laboratory parameters are considered safety and hence processed according to standard safety processing rules:

- Haematology (Haematocrit, Haemoglobin, Red blood cells (RBC) / Erythrocytes, White Blood Cells (WBC) / Leukocytes, Platelet Count / Thrombocytes)

- Clinical Chemistry (Albumin, Alkaline phosphatase, ALT (alanine transaminase), AST (aspartate transaminase), Bilirubin total, Creatinine, eGFR, Creatine kinase, Troponin, Lactate dehydrogenase, Glucose, Potassium, Sodium, Calcium, Magnesium, Urea (BUN), NT-proBNP

For continuous safety laboratory parameters standardized values will be derived as well as the differences to baseline. Laboratory parameters will be shown in SI units.

Laboratory values will be compared to their reference ranges and frequency tables will be provided for the number of patients within and outside the reference range at baseline, each visit and the last measurement on treatment. Descriptive statistics will be provided by treatment group for baseline, each visit and last value on-treatment and for changes from baseline to each respective visit and last value on treatment. The laboratory parameters will be summarized by day of assessment.

7.8.3 Vital signs

The following vital signs will be descriptively analysed: systolic and diastolic blood pressure, body temperature, respiratory rate and pulse rate. Both absolute values and change from baseline are planned for this section of the report.

7.8.4 ECG

Not applicable.

7.8.5 Others

7.8.5.1 Pharmacokinetic analysis

Descriptive statistics of all PK parameters will be presented and will be performed on the PKS as outlined in Section 6.3. In particular, the parameters / endpoints are:

- Css,1 (plasma concentration of alteplase at steady state of 2 hour infusion)
- Css,2 (plasma concentration of alteplase at steady state of long-term infusion)

Individual endogenous Tissue Plasminogen activator (TPA) will be determined for each patient at baseline using the first PK sample. Baseline levels will then be subtracted from all further concentration measurements (second and third samples), for each patient, in order to determine the Css,1 and Css,2 endpoints. In case the resulting concentration value is negative, it will then be set to "BLQ".

As outlined in CTP Version 3 Section 7.2.4, a population PK analysis is planned to be conducted if sufficient data are available. The objectives of the population PK analysis are:-

1) to compare the alteplase concentrations in study 0135-0347 to PK simulations based on a previously developed population PK model for patients with acute myocardial infarction (AMI)
2) to derive model-based area under the curve (AUC) estimates for patients with ARDS based on the sparse PK measurements of study 0135-0347

Dataset preparation
Alteplase plasma concentrations, sampling times, infusion times, and covariates (e.g., age, weight, sex, and race) will be assembled and formatted for analysis. Detailed dataset specifications will be provided in a separate document.

Handling of missing data
Handling of missing data will be described in the dataset specification document.

Handling of outliers
Alteplase concentrations and covariate values will be included in the analysis whenever possible. However, for methods based on least-squares estimation and normal theory it is not good practice to include extreme values. Thus, outlying data points in the dependent variable may be excluded if they are infrequent, occur randomly and are spurious. Furthermore, it may be justified to exclude outlying individuals from model development. Also, extreme covariate values might be excluded. If more than 5% of alteplase plasma concentrations are excluded, a sensitivity analysis will be performed to evaluate the impact of the excluded data points on the final model. All outliers which are excluded from the analysis will be documented along with reasons for their exclusion.

Pharmacokinetic modeling strategy

In the following the framework of the PK modelling strategy is outlined, this may be adjusted as deemed necessary.

The pharmacokinetics of alteplase follows a 3-compartment distribution. A 3-compartment model cannot be developed using the information from study 0135-0347 alone as only sparse PK data is collected in a subset of the study population. Therefore, a previously developed population PK model for alteplase will be used to inform the current analysis. The population PK model for patients with AMI which is based on studies 0135.30 and 0135.53 is the preferred starting model. The pharmacokinetics of alteplase in these AMI studies were best described by a linear 3 compartment model. A baseline t-PA concentration was estimated to account for endogenous t-PA in plasma. Weight was included as covariate affecting baseline t-PA concentration and clearance of alteplase. Age was included as covariate affecting clearance of alteplase.

First, the alteplase plasma concentrations in study 0135-0347 will be compared to PK model simulations. Simulations will be performed using the previously developed population PK model for alteplase in patients with AMI. In the simulations, inter-individual and residual variability will be considered. The alteplase plasma concentration-time profiles will be simulated 1,000 times for each subject in study 0135-0347 assuming its actual infusion regimen, sampling scheme and weight. The simulated alteplase plasma concentrations at the different time points will be used to calculate the median concentration-time profile as well as it's 90% prediction interval. This simulation results will be graphically compared to the observed data in study 0135-0347.

Given that the model-simulated plasma PK is in the same range as the observations, it will be further investigated whether the population PK model for patients with AMI can describe the individual alteplase concentrations in ARDS patients. Individual post-hoc estimates will be derived for study 0135-0347 using the parameters of the population PK model for patients with AMI. The distribution of the post-hoc estimates around the typical parameters and the description of the individual plasma concentrations will be investigated.

Provided that the population PK model for patients with AMI successfully describes the individual profiles of patients with ARDS, the individual PK parameters will be determined. In addition, AUC will be derived within NONMEM® from the individual PK parameters considering the patients' actual alteplase doses, infusion regimen, and covariates. In case, the structural PK model of patients with AMI cannot describe the PK of patients with ARDS, necessary adjustments (e.g. re-estimation of clearance) might be implemented into the model.

The population PK model for patients with AMI comprises the impact of weight and age on the PK of alteplase. Assessment of any remaining trends for weight, age, sex, and race will be conducted e.g. by graphical inspection plotting empirical Bayes estimate versus covariate values and conditional weighted residuals (CWRES) versus covariate values.

Inter-subject variability will be modeled using exponential random effect models. Residual variability will be modeled starting with the same error model structure as in patients with

AMI; adjustments will be made if necessary. The set of inter individual (η) and residual (ε) variability values are assumed to be symmetrically distributed around 0 with variances ω^2 and σ^2, respectively.

Evaluation

If model development is necessary, objective function values (for nested models) as well as the criteria listed below will be considered:

- A "successful minimization" statement by the NONMEM® program at the end of the estimation step.
- The final parameter estimates have a number of significant digits of 3 for all θ's.
- Estimates of θ's not close to a boundary.
- Correlation of the uncertainty of the estimates of fixed effect parameters < 0.95 or >-0.95.
- No significant trends in the basic goodness-of-fit plots (predictions (PRED), individual predictions (IPRED) vs. DV)
- Residuals (CWRES) scatter randomly and uniformly around zero when plotted against population prediction and time.
- ETAs are normally distributed around 0.
- No significant model misspecification in the individual predicted concentration-time profiles when compared to the observed profiles.

If a model is accepted as final model, although one or more of the criteria above are not fulfilled, the reasons for its acceptance need to be given in the report.

If model development is necessary, the final model will be evaluated using a prediction corrected visual predictive check (pcVPC, 13). A visual predictive check shows the ability of the model to simulate data that are similar to the observed data that were used for model development. The concentration-time profiles will be simulated 1,000 times using the same actual doses, infusion regimen, subjects' covariates, and study design as in the analysis data set. In order to adjust for the differences in e.g. actual doses, a pcVPC will be performed in which the observed and simulated DV will be normalized based on the typical population prediction.

8. TIMEPOINT OF RELEASE OF TREATMENT INFORMATION

8.1 HANDLING OF INDIVIDUAL PATIENT TREATMENT INFORMATION

This is an open-label study, therefore no blinding will apply for patients, investigators and personnel at the site involved in trial conduct. The access to the (planned) randomisation code list will be kept restricted until it is released for analysis.

The treatment information for an individual patient will be available in the trial database after randomisation.

Select data derivations, mostly related to safety endpoints, are treatment dependent. Therefore, the actual treatment codes available on the database will be used to create these treatment dependent data derivations, ongoing throughout the duration of the trial.

8.2 HANDLING OF AGGREGATED TREATMENT INFORMATION

While the study is in progress, access to tabular results of study outcomes by treatment allocation will not be made available to the patients, investigators and personnel at the site, to the trial statistician, clinical team, or members of the steering committee (unless the DMC advises otherwise). The DMC and DMC statisticians will be unblinded.

Ongoing tabular results of study outcomes will be produced using mock (dummy) treatment codes, throughout the duration of the trial, and until database snapshot.

At the timepoint of the database snapshot, following completion of day 28 visit for all patients, the actual treatment codes will be used to unblind the data for analysis.

9. REFERENCES

1.	*CPMP/ICH/363/96:* "Statistical Principles for Clinical Trials", ICH Guideline Topic E9, Note For Guidance on Statistical Principles for Clinical Trials, current version.
2.	0135-0347 Clinical Trial Protocol Version 3 (c32434345-03)
3.	WHO Working Group on the Clinical Characterisation and Management of COVID-19 infection. A minimal common outcome measure set for COVID-19 clinical research. Lancet Infectious Diseases, Published Online June 12, 2020, doi: 10.1016/S1473-3099(20)30483-7; 2020. [R20-2002]
4.	Schulman S, Kearon C. Subcommittee on Control of Anticoagulation of the Scientific and Standardization Committee of the International Society on Thrombosis and Haemostasis. Definition of major bleeding in clinical investigations of antihemostatic medicinal products in non-surgical patients. J Thromb Haemost. 2005. 3: 692-694. [R05-0344]
5.	*001-MCS-40-413:* Identify and Manage Important Protocol Deviations (iPD)", current version, Group "Clinical Operations", IDEA for CON.
6.	*001-MCS-40-135_RD-01:* "Integrated Quality and Risk Management Plan", current version, Group "Clinical Operations", IDEA for CON.
7.	*001-MCG-156*: "Handling and summarization of adverse event data for clinical trial reports and integrated summaries", current version; IDEA for CON.
8.	*001-MCG-159_RD-06*: "Standard table shells for inferential and descriptive Company Standard Displays (CSD-Catalogue)", current version; IDEA for CON.
9.	*001-MCG-159*: "Reporting of Clinical Trials and Project Summaries", current version; IDEA for CON.
10.	*001-MCG-156*: "Handling and summarization of adverse event data for clinical trial reports and integrated summaries", current version; IDEA for CON.
11.	REGULATION (EU) No 536/2014 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 16 April 2014 on clinical trials on medicinal products for human use, and repealing Directive 2001/20/EC, European Commission webpage.
12.	*001-MCG-157*: "Handling, Display and Analysis of Laboratory Data", current version; IDEA for CON.
13.	Bergstrand M, Hooker AC, Wallin JE, Karlsson MO Prediction-corrected visual predictive checks for diagnosing nonlinear mixed-effects models. AAPS J 2011 ; 13(2) ; 143-151. [R13-0300]

11. HISTORY TABLE

Table 11: 1 History table

Version	Date	Author	Sections changed	Brief description of change
1	**26-OCT-2021**	[redacted]	None	This is the final TSAP