

Clinical Trial Protocol

Document Number:		c32434345-02
EudraCT No. EU Trial No.	2020-002913-16	
BI Trial No.	0135-0347	
BI Investigational Medicinal Product(s)	Alteplase (recombinant tissue-type plasminogen activator, rt-PA)	
Title	The TRISTARDS trial - ThRombolysIS Therapy 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.	
Lay Title	The TRISTARDS trial - ThRombolysIS Therapy for ARDS A study to test whether different doses of alteplase help people with severe breathing problems because of COVID-19.	
Clinical Phase	IIb/III	
Clinical Trial Leader	Zuzana Holländerova, RNDr. Boehringer-Ingelheim RCV Dr. Boehringer-Gasse 5-11 A-121 Vienna, Austria Phone: +43 (1) 801 05-7730 Fax: +43 (1) 801 05-2375	
Coordinating Investigator	Prof. Markus Weigand Universitätsklinikum Heidelberg Im Neuenheimer Feld 110 69120 Heidelberg, Germany Tel. +49 6221 56-6350 Fax. +49 6221 56-5345	
Version and Date	Version: 2.0	Date: 22 Oct 2020
Page 1 of 95		
<p style="text-align: center;">Proprietary confidential information.</p> <p>© 2020 Boehringer Ingelheim International GmbH or one or more of its affiliated companies. All rights reserved. This document may not - in full or in Part - be passed on, reduced, published or otherwise used without prior written permission.</p>		

CLINICAL TRIAL PROTOCOL SYNOPSIS

Company name	Boehringer Ingelheim (BI)
Protocol date	21 July 2020
Revision date	21 Oct 2020
BI trial number	0135-0347
Title of trial	<p>The TRISTARDS trial - ThRombolysIS Therapy for ARDS</p> <p>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 in addition to standard of care (SOC) compared with SOC alone, in patients with acute respiratory distress syndrome (ARDS) triggered by COVID-19.</p>
Coordinating Investigator	<p>Prof. Markus Weigand Universitätsklinikum Heidelberg Im Neuenheimer Feld 110 69120 Heidelberg, Germany Tel. +49 6221 56-6350 Fax. +49 6221 56-5345</p>
Trial site(s)	Multi-centre trial conducted in approximately 14 countries
Clinical phase	IIb/III
Trial rationale	<p>ARDS is an acute illness of various etiologies (e.g. infections such as COVID-19) and is one of the major causes of mortality in COVID-19. ARDS due to COVID-19 is associated with fibrin wall thickening and micro-/macrovascular thrombi formation, while at the same time fibrinolysis is impaired. There is accumulating data from animal studies, historical trials in ARDS and clinical cases / case series reported during the COVID-19 pandemic that application of thrombolytic drugs are associated with increased oxygenation and improved clinical outcomes of ARDS, as a result of clearance of fibrin from the alveolar space and lysis of microclots. Alteplase (recombinant tissue-type plasminogen activator) may therefore have a role in the treatment of ARDS associated with COVID-19.</p>
Trial objective(s)	To evaluate the efficacy and safety of intravenous alteplase in ARDS triggered by COVID-19.
Trial endpoints	<p>The same primary endpoint will be evaluated in Part 1 and in Part 2 as follows:</p> <ul style="list-style-type: none"> 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.

	<p>The same secondary endpoints to evaluate safety and efficacy in this setting will be evaluated in Part 1 and in Part 2, as follows:</p> <ul style="list-style-type: none"> • All cause mortality at Day 28 • Number of ventilator-free days from start of treatment to Day 28 • Improvement of Sequential (sepsis-related) Organ Failure Assessment (SOFA) score by ≥ 2 points from baseline to end of Day 6 • Major bleeding events (MBE) (according to International Society on Thrombosis and Haemostasis [ISTH] definition) until Day 6 • Daily average PaO₂/FiO₂ ratio (or inferred PaO₂/FiO₂ ratio from SpO₂) change from baseline to Day 6 • All-cause mortality or on mechanical ventilation at Day 28 <p>With regard to each efficacy and safety endpoint, the term "baseline" refers to the last observed measurement prior to randomisation for all patients.</p>
Trial design	<p>Open-label, randomised, sequential parallel design comparison of:</p> <p>Part 1: two treatment arms and a standard of care (SOC) group (1:1:1 randomisation)</p> <p>Part 2: one treatment arm and a standard of care group (2:1 randomisation)</p>
Total number of patients randomised	<p>Part 1: approximately 60 patients Part 2: approximately 210 patients</p> <p>Randomisation will be stratified by type of ventilation support (invasive mechanical / non-invasive)</p>
Number of patients on each treatment	<p>Part 1: approximately 20 patients in SOC arm and 20 patients in each treatment arm Part 2: approximately 70 patients in SOC arm and 140 in treatment arm</p>
Diagnosis	<p>ARDS due to COVID-19</p>

<p>Main in- and exclusion criteria</p>	<p>Eligibility criteria for Parts 1 and 2 are the identical. Patients participating in Part 1 are not eligible for Part 2.</p> <p>Main criteria for inclusion:</p> <ol style="list-style-type: none"> 1. Age \geq 18 years (or above legal age, e.g. UK \geq16 years) 2. ARDS with PaO₂*/FiO₂ ratio >100 and ≤ 300 , either on non-invasive ventilator support, OR on mechanical ventilation (<48 hours since intubation), <ul style="list-style-type: none"> • with bilateral opacities in chest X-ray or CT scan (not fully explained by effusions, lobar/lung collapse, or nodules) • with respiratory failure (not fully explained by cardiac failure/fluid overload) <p>*or estimation of PaO₂/FiO₂ from pulse oximetry (SpO₂/FiO₂)</p> 3. SARS-CoV-2 positive (laboratory-confirmed RT-PCR test) 4. Fibrinogen level \geq upper limit of normal 5. D-Dimer \geq 3-fold of upper limit of normal (ULN) according to local laboratory 6. Signed and dated written informed consent in accordance with ICH Good Clinical Practice (GCP) and local legislation prior to admission to the trial. <p>Main criteria for exclusion:</p> <ol style="list-style-type: none"> 1. Massive confirmed pulmonary embolism (PE) with haemodynamic instability at trial entry 2. Patients on mechanical ventilation for longer than 48 hours 3. Chronic pulmonary disease i.e. with known forced expiratory volume in 1 second (FEV₁) $<50\%$ requiring home oxygen, or oral steroid therapy or hospitalisation for exacerbation within 12 months, or significant chronic pulmonary disease in the Investigator's opinion, or primary pulmonary arterial hypertension 4. Has a Do-Not-Intubate (DNI) or Do-Not-Resuscitate (DNR) order 5. In the opinion of the investigator, is not expected to survive for > 48 hours after admission <p>Further criteria apply to exclude patients at higher risk of bleeding.</p>
<p>Test product(s)</p>	<p>Alteplase, recombinant tissue-plasminogen activator, rt- PA, powder and solvent for infusion</p>

dose	<p>Part 1:</p> <p>Dosing regimen A: Initial i.v. infusion of alteplase 0.3 mg/kg over 2 hours (Day 1) immediately followed by daily i.v. infusion of 0.02 mg/kg/hour over 12 hours (starting on Day 1 and up to Day 5 days at maximum)</p> <p><i>One optional additional i.v. infusion of 0.3 mg/kg over 2 hours can be given once on Days 2 to 5 in case of clinical worsening (as per investigator judgement)</i></p> <p>OR:</p> <p>Dosing regimen B: Initial i.v. infusion of alteplase 0.6 mg/kg over 2 hours (Day 1) immediately followed by daily i.v. infusion of 0.04 mg/kg/hour over 12 hours (starting on Day 1 and up to Day 5 at maximum)</p> <p><i>One optional additional i.v. infusion of 0.6 mg/kg over 2 hours can be given once on Days 2 to 5 in case of clinical worsening (as per investigator judgement)</i></p> <p>Part 2: One dosing regimen will be selected dependent on Part 1 results</p>
mode of administration	Intravenous (i.v.)
Comparator product(s)	N/A
dose	N/A
mode of administration	N/A
Duration of treatment	Up to a maximum of 5 days
Statistical methods	<p>The trial consists of two parts which, for the purpose of statistical analyses, will be treated independently. Part 1 will be descriptive, and Part 2 will be confirmatory. In both parts, the respective full analysis set (FAS) will be used and the same primary endpoint for both Parts 1 and 2 will apply. Only patients randomised into Part 1 will be included in the analyses of Part 1, and only patients randomised into Part 2 will be included in the confirmatory analyses of Part 2.</p> <p>The primary endpoint for Part 1 will be analysed in a similar manner to Part 2, albeit in Part 1 this will be exploratory in nature.</p> <p>Following the completion of Part 1, the DMC will review the unblinded efficacy and safety data to make a recommendation for proceeding into Phase III (Part 2). This recommendation will be with respect to selecting one dosing regimen for Phase III.</p> <p>For Part 2, the primary analysis of the time to clinical improvement or hospital discharge up to Day 28 will be analysed using the Cox proportional hazard model. This endpoint is defined as the time from randomisation to either an improvement of two points on the 11-point</p>

	<p>WHO Clinical Progression Scale or discharge from the hospital, whichever comes first. Patients will be censored at Day 28 if they die prior to Day 28. If they receive bail-out therapy, they will be censored on the day of receiving the bail-out therapy. Patients who have not had clinical improvement by Day 28 will be censored on Day 28. The Cox proportional hazard model will be used to estimate the hazard ratio, the 95% confidence interval and p-value. The model will contain fixed effects for treatment, type of ventilation, baseline status on the 11-point WHO Clinical Progression Scale and age.</p> <p>Descriptive statistics will be used to analyse all other endpoints.</p>
--	--

FLOW CHART

Trial Periods	Screening	Intravenous open-label treatment					Follow-Up			
Visit number	1	2a	2b	2c	2d	2e / EOT	3	4	5	6 / EOS
Day	0	1	2	3	4	5	6	7	28	90
Hours	-24 - 0	0-14	26-38	50-62	74-86	98-110	134			
Time windows [hrs, if not specified otherwise]		+6	±2	±2	±2	+6	±12	+1 day	+2 days	±7 days
Informed consent (separate IC for patients participating in PK sampling)	X									
Demographics	X									
Medical history	X									
Physical examination	X					X			X	
Weight	X									
Height	X									
Vital signs ¹	X	X	X	X	X	X	X	X	X	
12-lead ECG ²	X	(X only needed in case of clinical event) ²							X	
Review of in-/exclusion criteria	X									
IRT call	X	X				X				
Randomisation ³		X								
Dispense trial drugs		X				X				
Administer trial drugs ⁴		X	X	X	X	X				
PK Sampling ⁵		X								
Laboratory Tests	X			X		X	X ⁶		X	
D-dimer assessment ⁷	X			X		X		X	X	
Fibrinogen, aPTT, antithrombin and anti-Factor Xa assessment ⁸	X	X	X	X	X	X		X	X	
PaO ₂ /FiO ₂ ratio (or inferred PaO ₂ /FiO ₂ ratio from SpO ₂) ⁹	X	X	X	X	X	X	X	X	X	
Intubation/Extubation status assessment	X	X	X	X	X	X	X	X	X	
Trial specific examinations: SOFA score ¹⁰	X			X		X	X			
Trial specific examinations: APACHE II score ¹⁰	X			X		X	X		X	
11-point WHO Clinical Progression Scale (see Section 5.1.1) ¹¹	X	X	X	X	X	X	X	X	X	
Pregnancy testing ¹²	X								X	
SARS-CoV-2 test ¹³	X								X	
All adverse events/SAE adverse events	X	X	X	X	X	X	X	X	X	

Trial Periods	Screening	Intravenous open-label treatment					Follow-Up			
Visit number	1	2a	2b	2c	2d	2e / EOT	3	4	5	6 / EOS
Day	0	1	2	3	4	5	6	7	28	90
Concomitant therapy	X	X	X	X	X	X	X	X	X	
Completion of patient participation ¹⁴										X
Vital status collection ¹⁵										X

Footnotes:

- Vital signs measurement shall include BP measurement, body temperature, respiratory rate and pulse rate.
- In addition to the scheduled ECGs (electrocardiograms), ECGs should be done and documented at any time of clinical event (e.g. acute event of arrhythmia, tachy- or bradycardia, angina, or MI).
- Randomisation of patients shall happen not later than 24 hours after the confirmation of the inclusion and exclusion criteria, and study drug administration shall start within 6 hours of the randomisation
- 12h infusion of Alteplase per day over up to 5 days followed by an interruption of approximately 12 h each day (Day 1 and any day of additional/optional i.v. infusion: 2 h i.v. infusion is followed by 12 h long-term i.v. infusion) . Does not apply for SOC treatment arm.
- PK samples collection (on a voluntary basis, Part 1 only): 1st sample: prior to first drug administration on Day 1 (Visit 2a pre-dose) (planned time: -1:00 h; time window -2:00 h to just prior drug administration), 2nd sample: at steady state of the initial i.v. infusion on Day 1 (Visit 2a post-dose initial i.v. infusion) (planned time 1:30 h; time window: between 1:00 h after start of initial i.v. infusion and just prior to end of initial i.v. infusion), 3rd sample: at steady state of the long-term i.v. infusion on Day 1 (Visit 2a post-dose long-term i.v. infusion) (planned time: 6:00 h; time window: between 2 h after start of long-term i.v. infusion and just prior to end of long-term i.v. infusion) For further details refer to [Section 5.3.1](#). Separate informed consent has to be signed before including patient in PK collection.
- On Day 6 only lab samples required for APACHE II, SOFA score and PaO₂/FiO₂ shall be collected .
- D-dimer levels shall be measured at Day 0 (screening), days 3, 5, 7 and 28
- During the treatment period (alteplase arm only), fibrinogen should be measured daily. The aPTT (for all patients on unfractionated heparin [UFH]) or anti-Xa (for all patients on low-molecular weight heparin [LMWH]) should be measured at least daily, preferably aPTT should be measured more often (more details see [Section 4.2.1](#))
- PaO₂/FiO₂ is only measured during hospital stay. The very first and last PaO₂/FiO₂ should preferably be obtained, when the patient is in a supine position. During the first 6 days and during the stay on ICU (whatever is longer), PaO₂/FiO₂ ratio should be measured at least three times daily in all patients, together with a recording of the patient's position.
- APACHE II score will be evaluated up to Day 28 and SOFA will be assessed until Day 6 including vital signs assessment and laboratory samples needed for score calculations. (please refer to [Appendix 10.2](#)). The Glasgow Coma Scale (GCS), used in the APACHE II calculation, is provided in [Appendix 10.1](#). If patient is discharged from hospital prematurely, last evaluation during hospital stay is expected at day of discharge from hospital.
- WHO clinical progression scale should be collected daily during hospital stay.
- Only applicable for women of childbearing potential. Urine pregnancy test will be performed at screening and at Visit 5. Study drug should only be administered in case of a negative test result.
- SARS-CoV-2 laboratory confirmation (RT-PCR test) as a pre-requisite before or at screening. Another test for SARS-CoV-2 should be performed to check for recovery or persistence of infection at Visit 5. The choice of laboratory tests should follow local practice.
- Patients who discontinue trial treatment prematurely should still follow the trial schedule until Visit 6/EOS, if possible, please see [Section 3.3.4](#).

15. Patients will be followed-up until Day 90 for vital status collection. No onsite visit is required, any locally approved way of obtaining vital status information is accepted.

TABLE OF CONTENTS

TITLE PAGE	1
CLINICAL TRIAL PROTOCOL SYNOPSIS	2
FLOW CHART	7
TABLE OF CONTENTS	10
ABBREVIATIONS	13
1. INTRODUCTION.....	16
1.1 MEDICAL BACKGROUND.....	16
1.2 DRUG PROFILE	17
1.3 RATIONALE FOR PERFORMING THE TRIAL	19
1.4 BENEFIT - RISK ASSESSMENT.....	20
1.4.1 Benefits.....	20
1.4.2 Risks	22
1.4.3 Discussion.....	26
2. TRIAL OBJECTIVES AND ENDPOINTS.....	27
2.1 MAIN OBJECTIVES, PRIMARY AND SECONDARY ENDPOINTS.....	27
2.1.1 Main objectives.....	27
2.1.2 Primary endpoint	27
2.1.3 Secondary endpoints	27
2.2 FURTHER OBJECTIVES AND FURTHER ENDPOINTS	27
2.2.1 Further objectives	27
2.2.2 Further endpoints	28
3. DESCRIPTION OF DESIGN AND TRIAL POPULATION	29
3.1 OVERALL TRIAL DESIGN.....	29
3.2 DISCUSSION OF TRIAL DESIGN, INCLUDING THE CHOICE OF CONTROL GROUP(S)	30
3.3 SELECTION OF TRIAL POPULATION	31
3.3.1 Main diagnosis for trial entry	32
3.3.2 Inclusion criteria	32
3.3.3 Exclusion criteria	33
3.3.4 Withdrawal of patients from treatment or assessments.....	34
3.3.4.1 Discontinuation of trial treatment	34
3.3.4.2 Withdrawal of consent to trial participation	35
3.3.4.3 Discontinuation of the trial by the sponsor	36
4. TREATMENTS.....	37
4.1 INVESTIGATIONAL TREATMENTS	37
4.1.1 Identity of the Investigational Medicinal Products.....	37
4.1.2 Selection of doses in the trial and dose modifications.....	39
4.1.3 Method of assigning patients to treatment arms.....	40
4.1.4 Drug assignment and administration of doses for each patient.....	40

4.1.5	Blinding and procedures for unblinding.....	42
4.1.5.1	Blinding.....	42
4.1.5.2	Unblinding and breaking the code	42
4.1.6	Packaging, labeling, and re-supply.....	42
4.1.7	Storage conditions	42
4.1.8	Drug accountability.....	42
4.2	OTHER TREATMENTS, EMERGENCY PROCEDURES, RESTRICTIONS	43
4.2.1	Other treatments and emergency procedures	43
4.2.2	Restrictions	45
4.2.2.1	Restrictions regarding concomitant treatment	45
4.2.2.2	Restrictions on diet and life style.....	45
4.2.2.3	Contraception requirements	45
4.3	TREATMENT COMPLIANCE	46
5.	ASSESSMENTS.....	47
5.1	ASSESSMENT OF EFFICACY	47
5.1.1	WHO Clinical Progression Scale.....	47
5.1.2	Ventilator-free days, days in ICU.....	48
5.1.3	PaO ₂ /FiO ₂ ratio.....	48
5.1.4	SOFA Score	48
5.1.5	APACHE II Score	48
5.2	ASSESSMENT OF SAFETY.....	48
5.2.1	Physical examination	48
5.2.2	Vital signs.....	49
5.2.3	Safety laboratory parameters	49
5.2.4	Electrocardiogram	51
5.2.5	Other safety parameters.....	51
5.2.5.1	Bleeding events	51
5.2.6	Assessment of adverse events.....	52
5.2.6.1	Definitions of adverse events.....	52
5.2.6.2	Adverse event collection and reporting	55
5.3	DRUG CONCENTRATION MEASUREMENTS AND PHARMACOKINETICS	56
5.3.1	Assessment of pharmacokinetics	56
5.3.2	Methods of sample collection	56
5.3.3	Analytical determinations	57
5.4	ASSESSMENT OF BIOMARKERS	57
5.5	BIOBANKING	57
5.6	OTHER ASSESSMENTS.....	57
5.7	APPROPRIATENESS OF MEASUREMENTS	57
6.	INVESTIGATIONAL PLAN.....	58
6.1	VISIT SCHEDULE.....	58
6.2	DETAILS OF TRIAL PROCEDURES AT SELECTED VISITS	58
6.2.1	Screening and run-in periods.....	59

6.2.2	Treatment periods.....	59
6.2.3	Follow-up period and trial completion.....	60
7.	STATISTICAL METHODS AND DETERMINATION OF SAMPLE SIZE	61
7.1	NULL AND ALTERNATIVE HYPOTHESES	61
7.2	PLANNED ANALYSES	61
7.2.1	General considerations	61
7.2.2	Primary endpoint analyses	62
7.2.3	Secondary endpoint analyses	63
7.2.4	Further endpoint analyses.....	63
7.2.5	Safety analyses.....	63
7.2.6	Interim Analyses	64
7.3	HANDLING OF MISSING DATA	64
7.4	RANDOMISATION	64
7.5	DETERMINATION OF SAMPLE SIZE	65
8.	INFORMED CONSENT, TRIAL RECORDS, DATA PROTECTION, PUBLICATION POLICY, AND ADMINISTRATIVE STRUCTURE	67
8.1	TRIAL APPROVAL, PATIENT INFORMATION, INFORMED CONSENT	67
8.2	DATA QUALITY ASSURANCE	68
8.3	RECORDS	68
8.3.1	Source documents	69
8.3.2	Direct access to source data and documents.....	70
8.3.3	Storage period of records	70
8.4	EXPEDITED REPORTING OF ADVERSE EVENTS	70
8.5	STATEMENT OF CONFIDENTIALITY AND PATIENT PRIVACY	70
8.5.1	Collection, storage and future use of biological samples and corresponding data	71
8.6	TRIAL MILESTONES.....	71
8.7	ADMINISTRATIVE STRUCTURE OF THE TRIAL	72
9.	REFERENCES.....	74
9.1	PUBLISHED REFERENCES.....	74
9.2	UNPUBLISHED REFERENCES.....	79
10.	APPENDICES	81
10.1	GLASGOW COMA SCALE.....	81
10.2	SOFA AND APACHE II SCORES	82
10.3	TABLE TO DERIVE PAO ₂ /FIO ₂ RATIO FROM SPO ₂	84
10.4	POTENTIAL MODIFICATION OF TRIAL CONDUCT IN CASE OF RESTRICTIONS DUE TO COVID-19.....	86
10.5	ALTEPLASE DOSING TABLE.....	88
11.	DESCRIPTION OF GLOBAL AMENDMENT(S)	92
11.1	GLOBAL AMENDMENT 1	92

ABBREVIATIONS

ACE	angiotensin-converting enzyme
AE	adverse event
ALCOA	attributable, legible, contemporaneous, original, accurate
ALT	alanine aminotransferase
APACHE	Acute Physiologic And Chronic Health Evaluation
aPTT	activated partial thromboplastin time
ARDS	acute respiratory distress syndrome
ASA	acetylsalicylic acid
AST	aspartate aminotransferase
BI	Boehringer Ingelheim
BP	blood pressure
COVID-19	coronavirus disease 2019
CRA	Clinical Research Associate
CRF	Case report form, paper or electronic (sometimes referred to as “eCRF”)
CRI	Constant rate infusion
CRO	Contract research organisation
CT Leader	Clinical Trial Leader
CTP	clinical trial protocol
CTR	clinical trial report
DBL	database lock
DMC	Data Monitoring Committee
EC	Ethics Committee
ECG	electrocardiogram
ECMO	extracorporeal membrane oxygenation
eCRF	electronic case report form
EOT	End of treatment
EOS	End of trial
EudraCT	European Clinical Trials Database
FAS	Full analysis set

FEV ₁	forced expiratory volume in 1 second
FiO ₂	fraction of inspired oxygen
FPI	First patient in
GI	gastrointestinal
GCP	Good Clinical Practice
GPIIb/IIIa	glycoprotein IIb/IIIa
GUSTO	Global Utilization of Streptokinase and Tissue Plasminogen Activator for Occluded Coronary Arteries
HIT	Heparin-induced thrombocytopenia
i.v.	intravenous
IB	Investigator's Brochure
ICU	intensive care unit
IMP	Investigational medicinal product
INR	International normalised ratio
IRB	Institutional Review Board
IRT	Interactive response technology
ISF	Investigator site file
ISTH	International Society on Thrombosis and Haemostasis
kDa	kilodalton
LMWH	low-molecular weight heparin
LPLT	Last patient last treatment
MBE	Major bleeding events
MedDRA	Medical Dictionary for Drug Regulatory Activities
MI	myocardial infarction
MRT	magnetic resonance tomography
OE	Outcome event
PAI-1	plasminogen activator inhibitor Type 1
PaO ₂	partial oxygen pressure in arterial blood
PaO ₂ /FiO ₂	Oxidation Index (Horowitz Index)
PE	pulmonary embolism
PK	pharmacokinetics
PPS	Per Protocol Set
rt-PA	recombinant tissue-type plasminogen activator

RBC	red blood cell
REP	residual effect period
RRT	renal replacement therapy
RS	Randomised Set
SARS-CoV-2	severe acute respiratory syndrome coronavirus 2
SAE	serious adverse event
SC	Steering Committee
SGPT	serum glutamic-pyruvic transaminase
SGOT	serum glutamic-oxaloacetic transaminase
SOC	Standard of Care
SmPC	Summary of Product Characteristics
SOFA	Sequential (sepsis-related) Organ Failure Assessment
SOP	standard operating procedure
SpO ₂	Oxygen saturation
SUSAR	Suspected unexpected serious adverse reactions
t _{1/2}	Half life time
TS	Treated Set
TSAP	Trial Statistical Analysis Plan
UFH	unfractionated heparin
ULN	upper limit of normal
WBC	white blood cell
WHO	World Health Organisation

1. INTRODUCTION

1.1 MEDICAL BACKGROUND

The current knowledge of COVID-19 is evolving. In 2019, an outbreak of acute respiratory illness emerged. In February 2020, the International Committee on Taxonomy of Viruses announced “severe acute respiratory syndrome coronavirus 2” (SARS-CoV-2) as the name of the new virus, and the WHO designated the disease “coronavirus disease 2019,” or COVID-19. The WHO declared COVID-19 a public health emergency in January 2020 and a pandemic in March 2020. During this pandemic, cases have grown exponentially around the world.

The infection is a self-limiting illness in most people, but in a significant proportion of the population, particularly the elderly, results in ARDS, with severe progressive pneumonia, multiorgan failure, and death [[R20-1310](#), [R20-1309](#)]. Experience suggests that 5 to 16% of patients hospitalised with COVID-19 will undergo prolonged intensive care and 50 to 70% thereof require mechanical ventilation [[R20-1310](#), [R20-1309](#)]. The mortality rate is 25 to 60% in severely affected patients with ARDS with the current standard of care [[R20-1652](#)].

COVID-19 and other infections are associated with ARDS. The exact mechanism contributing to a rapid lung injury in patients with ARDS is not fully understood, but diffuse alveolar damage typically marks the onset of ARDS. In early phases, epithelial and endothelial injury is characterized by loss of integrity of the barrier with plasma rich in albumin and fibrinogen and cellular debris exuding into the alveolar spaces which probably accentuates the inflammatory process [[R20-1654](#)]. A procoagulant state stems from increased tissue factor expression and suppression of fibrinolytic activity due to a rise in plasminogen activator inhibitor Type 1 (PAI-1) [[P94-80931](#)]. This leads to the formation of microthrombi in the lungs, as demonstrated by autopsy, further compromising gas exchange. [[R20-1366](#), [R20-1643](#), [R20-1952](#)].

Additionally, fibrin formation within the lungs starts a vicious cycle in which leukocytes localized to the clot through integrin binding sites within the fibrinogen molecule exacerbate inflammation and fibrinolysis [[R20-1653](#)]. Emerging evidence indicates that activation of coagulation as demonstrated by elevated D-dimer and fibrin degradation product levels are associated with poorer outcomes in novel coronavirus patients [[R20-1655](#)].

Current treatment standards for COVID-19 are emerging. Current treatment may include supportive measures applied in hospital, specifically on an intensive care unit (ICU), such as for example the use of non-invasive or invasive ventilation, oxygen masks, haemodynamic support, if needed, sedation, as well as medical therapies commonly used in patients suffering from ARDS or its complications. Standard of care follows the standard therapies established locally at the study site.

ARDS has no effective specific treatment besides supportive care, which includes conservative fluid strategies for patients, early empirical antibiotics for suspected bacterial

co-infection, the use of ventilation strategies encompassing low tidal volumes that limit trans-pulmonary pressures, and prone positioning in severe pulmonary disease [[R20-1647](#)].

As an established thrombolytic therapy, alteplase may have a role in targeting the coagulation and fibrinolytic systems to improve the treatment and possibly outcome of ARDS, an approach that has been proposed for at least the past two decades [[R20-1650](#)]. In particular, the use of plasminogen activators to limit ARDS progression and reduce ARDS-induced death has received strong support from animal models and human clinical trials. A meta-analysis of 22 preclinical studies in different species showed a significant reduction in mortality when using fibrinolytics via different routes, and conclude that fibrinolytic therapy may be an effective pharmaceutical strategy for ARDS in animal models [[R20-1656](#)].

A case series of five patients with COVID-19-associated ARDS receiving a bolus administration of alteplase (50 mg) and three patients with an additional re-bolus and concomitant heparin treatment and alteplase appeared to have an improved respiratory status following alteplase [[P20-04591](#)]. In a study with three severe COVID-19 related ARDS patient under mechanical ventilation, the administration of an intravenous bolus of alteplase (25 mg) followed by an infusion of 25 mg over 22 hours, led to a temporary improvement in their respiratory status (ranging from 38% to a ~100% improvement), with one of them having a sustained response, the two others having transient improvements [[P20-03126](#)]. A further study with four severe COVID-19 related patients with ARDS, under mechanical ventilation and a bolus-infusion concept (50 mg over 2 hours) showed a post treatment immediate improvement in gas exchange and/or hemodynamics [[P20-04704](#)]. The authors concluded that thrombolysis improved alveolar ventilation by restoring blood flow to previously occluded regions.

In other clinical trials, several therapeutic interventions for ARDS caused by SARS-CoV-2 are currently being evaluated, including anti-viral agents (e.g. remdesivir, European Clinical Trials Database [EudraCT] identifiers: 2020-000936-23, 2020-000842-32 and 2020-000841-15), anti-inflammatory agents (e.g. tocilizumab, clinicaltrials.gov: NCT04317092) as well as other approaches. Recent results showed that dexamethasone reduced deaths by one-third in ventilated patients and by one fifth in other patients receiving oxygen only. However, no benefit of dexamethasone was observed among patients who did not require respiratory support [[R20-1954](#)]. Yet there is a lack of controlled, randomised studies targeting the coagulation system, either by preventing coagulation or by supporting fibrinolysis, which is intrinsically linked to the inflammatory response [[P08-16186](#)].

1.2 DRUG PROFILE

Alteplase is a tissue plasminogen activator produced by recombinant DNA technology. Alteplase is a serine protease consisting of 527 amino acid residues with a molecular weight of 65 kDa. It shows extensive homology in primary structure with urokinase and has a catalytic site homologous with trypsin and other serine proteases. It contains an aminoterminal region that has a high degree of sequence homology with the "kringle" regions of plasminogen [[P96-2358](#)] and prothrombin. A kringle is a characteristic triple disulfide structure originally described in the "pro" fragment of prothrombin [[R96-2065](#)].

The aminoterminal region is responsible for the fibrin-specific activation of alteplase. The carboxyterminal end of the molecule contains a domain responsible for the protease activity of alteplase. The primary pharmacological effect of alteplase is activation of plasminogen by converting it to plasmin. This occurs extremely efficiently in the presence of fibrin. Fibrin has been shown to reduce the apparent Michaelis constant (K_m) for plasminogen activation by about 8-fold [P90-53645]. When administered into the systemic circulation at therapeutic concentrations, alteplase binds to fibrin in a thrombus. Subsequent conversion of fibrin-bound plasminogen to plasmin by alteplase results in the dissolution of thrombi.

Mode of action

Simplified, alteplase binds to fibrin clots and activates plasminogen, leading to the generation of plasmin and to the degradation of fibrin clots or blood coagulates. For a more detailed description of the alteplase profile, please refer to the current Investigator's Brochure (IB).

Key pharmacokinetic characteristics

The pharmacokinetic profile of alteplase after intravenous administration is well documented: alteplase is cleared rapidly from the circulating blood and metabolised mainly by the liver (plasma clearance 550 - 680 mL/min). The relevant plasma half-life $t_{1/2\alpha}$ is 4 – 5 minutes. This means that after 20 minutes less than 10% of the initial value is present in the plasma [U87-0935, U85-0697, U86-0354, U87-0368, P89-49823]. Alteplase was shown to have a high clearance rate and a removal from the circulation by specific uptake and degradation in the liver. The volume of distribution is confined to the blood volume and well-perfused tissues [P85-16126].

Drug interactions

No formal interaction studies with alteplase and medicinal products commonly administered in patients with acute myocardial infarction (MI) have been performed.

The risk of haemorrhage is increased if coumarine derivatives, oral anticoagulants, platelet aggregation inhibitors, unfractionated heparin or LMWH or active substances which interfere with coagulation are co-administered (before, during or within the first 24 hours after treatment with alteplase). Concomitant use of glycoprotein IIb/IIIa (GPIIb/IIIa) antagonists increases the risk of bleeding.

Concomitant treatment with angiotensin-converting enzyme (ACE) inhibitors may enhance the risk of suffering a hypersensitivity reaction.

Residual effect period

A single dose of 0.25 mg/kg alteplase (approximately 20 mg) infused over 30 minutes in healthy volunteers had no effect on coagulation parameters, indicative that systemic fibrinogenolysis was absent. This is consistent with the fibrin specificity and physiologic function of native alteplase [P89-49823]. For the residual amount remaining in deep compartments, a beta-half-life of about 40 minutes was measured.

The residual effect period (REP) of alteplase for this trial is defined as 7 days. This is the period after the last dose where pharmacodynamic effects are still likely to be present.

Data from non-clinical studies

For a more detailed description of the alteplase profile, please refer to the current Investigator's Brochure (IB) and to the Summary of Product Characteristics (SmPC)/local prescribing information.

Data from clinical studies

Alteplase is a well-established drug, first authorized in Europe in June 1987. It has been authorized worldwide in more than 100 countries.

Alteplase is currently indicated for the thrombolytic treatment of acute MI, acute massive PE, acute ischemic stroke, and occluded central venous access devices including those used for haemodialysis.

In a study including more than 40,000 patients with an acute MI (GUSTO) the administration of alteplase 100 mg over 90 minutes, with concomitant intravenous heparin infusion, led to a lower mortality after 30 days (6.3%) compared with the administration of streptokinase (1.5 million units over 60 minutes) with concomitant use of subcutaneous or intravenous heparin (7.3%) [[P93-77527](#)].

The results of ECASS III show a positive net-clinical benefit for alteplase in patients with stroke in the 3 to 4.5-hour time window [[P08-12177](#)]. Treatment administration in the ECASS III study was in line with the European SmPC for alteplase in its stroke indication. Further, pooled data demonstrating that the net-clinical benefit is no longer favorable for alteplase in the time window beyond 4.5 hours.

In patients with acute massive PE with haemodynamic instability thrombolytic treatment with alteplase leads to a fast reduction of the thrombus size and a reduction of pulmonary artery pressure. The approved alteplase dosage in haemodynamic-relevant pulmonary embolism is 10 mg as bolus followed by 90 mg over 2 hours (i.e. 100 mg in total given over 2 hours).

For a more detailed description of the alteplase profile, please refer to the current IB and for to the SmPC.

1.3 RATIONALE FOR PERFORMING THE TRIAL

The current study is needed to appropriately clinically evaluate the efficacy and safety of adding intravenous alteplase to standard medical care for patients with ARDS associated with COVID-19, and to determine the appropriate dose.

As an established thrombolytic therapy, alteplase may offer benefit for patients with ARDS associated with COVID-19 by targeting the fibrinolytic system [[R20-1760](#)]. Currently, such severely ill patients lack effective treatment besides supportive care. The results of Part 1 of the current study will provide proof of concept and establish the dose for Part 2, the confirmatory part of the study.

The current study is designed to enroll a study population that is in line with patients with ARDS, due to COVID-19, who are presently only receiving standard of care, i.e. supportive care. The study eligibility criteria, including age range, is anticipated to enroll a study population of severely ill patients with ARDS who have the capacity to benefit from study treatment.

In order to be able to address future scientific questions, patients will be asked to voluntarily donate samples for pharmacokinetic measurements (please see [Section 5.3.1](#)).

1.4 BENEFIT - RISK ASSESSMENT

1.4.1 Benefits

Alteplase is a currently approved, established fibrinolytic agent with a well-known safety and efficacy profile. Extensive experience with alteplase has been obtained over the past 30 years. Alteplase is licensed for the thrombolytic treatment of acute MI, massive PE with haemodynamic instability, and acute ischaemic stroke. In the current study, alteplase is expected to beneficially address the hypercoagulable state and compromised respiratory status associated with ARDS due to COVID-19. Alteplase can be administered in a regimen and approach that is suitable to address these clinical issues as well as to mitigate risks.

Dissolving extensive microvasculature thrombosis with potent and fast acting fibrinolytic substances such as alteplase is likely to be highly beneficial for patients with ARDS due to COVID-19, leading to improved oxygen exchange and end organ perfusion after alteplase application. Pre-clinical and previous clinical trial data in patients with ARDS suggest a positive effect of fibrinolytic compounds by improving oxygen exchange in these patients [[P00-10646](#), [P13-04526](#), [R20-1656](#), [P12-04032](#), [R20-1650](#), [R20-1934](#)]. Current pathology findings from deceased COVID-19 patients with ARDS show profound thrombotic obliterations of mainly (but not exclusively) end stream vasculature in lungs and other organs [[R20-1649](#), [P20-04704](#), [P20-04176](#), [R20-1851](#), [R20-1941](#), [R20-1804](#), [R20-1852](#)]. Additionally, histopathological evidence of widespread fibrin deposits in alveoli have been observed in these patients. Microvascular thrombosis in lung capillaries and fibrin deposits in the alveoli are considered to play a major role in the deterioration of lung function and potentially right ventricular function in patients with ARDS due to COVID-19. Impaired oxygen exchange in the alveoli (either affected by capillary thrombosis or fibrin deposition into alveoli) are key pathological processes, which make high flow oxygen support or even mechanical ventilation necessary in severely affected patients with ARDS due to COVID-19.

An additional complexity in severely affected patients with ARDS due to COVID-19 appears to be a prolonged thrombogenic status during active infection, which may lead to re-thrombosis and/or ongoing microvascular thrombosis contributing to associated organ dysfunctions. This prolonged thrombogenic status resulting in thrombosis-related organ dysfunction seems to be difficult to address with parenteral anticoagulants such as UFH or LMWH alone. UFH and LMWH will prevent or at least slow down the progression of (new) thrombus formation by its anticoagulant activity. UFH or PMWH will not directly dissolve existing thrombi. Adding alteplase to UFH or LMWH will additionally directly and immediately dissolve newly formed thrombi, which are re-occluding the microvasculature

despite UFH or LMWH treatment. Since alteplase has a short half-life of maximal 40 minutes, a bolus application will only be effective and beneficial for a short time point. Low dose constant rate infusion of alteplase is considered to be a dose regimen which addresses on a time-as-needed basis the ongoing re-formation of microthrombi during active SARS-CoV-2 infection [[U95-2167](#), [U99-1584](#)]. The combination of an initial 2 hours infusion of alteplase 0.3 mg/kg over 2 hours or 0.6 mg/kg over 2 hours combined with a constant rate infusion (CRI) of low dose alteplase 0.02 to 0.04 mg/kg/h over 12 hours is believed to dissolve the present thrombotic obstructions and the newly formed microthrombi in patients with ARDS due to COVID-19.

Alteplase can be given in combination with UFH or LMWH. This is routinely performed when applying treatment to acute MI patients or acute ischemic stroke patients. After initial alteplase treatment, the percutaneous intervention is always conducted under full anticoagulation.

Treating the patients with a moderate initial dose 0.3 mg/kg over 2 hours or 0.6 mg/kg over 2 hours combined with a constant rate infusion (CRI) of low dose alteplase 0.02 to 0.04 mg/kg/h over 12 hours is considered to reduce the risk of alteplase-related bleeding events, especially in comparison to repeated application of high alteplase doses (100 mg). Also a regular temporary interruption of CRI in contrast to continuous CRI over days is believed to reduce the bleeding risk by allowing the body to regenerate its plasma fibrinogen levels.

The risk of bleeding may be further mitigated because these patients are under close surveillance. Blood pressure, glucose levels, plasma fibrinogen levels are being closely monitored and intervention immediately applied in this setting as standard of care. Because alteplase has a very short half-life, interrupting the CRI will have an immediate effect on fibrinolysis. Bleeding is addressed rapidly either surgically or medically; e.g. with fresh frozen plasma (FFP), fibrinogen concentrate, or tranexamic acid.

While previous historical studies evaluating fibrinolytic therapy for treatment of ARDS used urokinase and streptokinase, the more contemporary approach to thrombolytic therapy involves the use of alteplase due to higher efficacy of clot lysis with comparable bleeding risk. This trial hypothesizes that administration of alteplase as a short term infusion (0.3 mg/kg over 2 hours or 0.6 mg/kg over 2 hours) plus low-dose long-term infusion for patients with COVID-19-related ARDS will improve pulmonary gas exchange and oxygenation via a decrease in pulmonary vascular microthrombi and lysis of fibrin in alveoli.

There is emerging evidence that patients with COVID-19-related ARDS have pulmonary thrombi in segmental and sub segmental vessels. Alteplase is proven to have a higher efficacy of clot lysis in arterial and venous thrombosis and might improve ventilation capacity (monitored by PaO₂/FiO₂ ratio) of COVID-19 related ARDS.

These patients appear to have a strong prothrombotic tendency and have a relative resistance to the action of anticoagulants. This condition is worsened due to an impairment of fibrinolysis which is addressed by the use of alteplase. While alteplase does not prevent

recurrence of thrombosis, the concomitant use of anticoagulation is intended to further prevent formation of new micro- or macroclots.

1.4.2 Risks

Bleeding events

Fibrinolytic treatment always harbours the risk of bleeding events. Depending on the anatomical location and severity of bleeding, the bleeding can be life threatening or even turn out fatal. The bleeding risk is potentially augmented when combining alteplase with anticoagulants or platelet inhibitor due to the combination of fibrinolysis, anticoagulation and/or platelet inhibition. The bleeding risk of alteplase also differs in relation to dose and rate. High doses of alteplase given at high rates increases bleeding risk. Low levels of fibrinogen (<100 mg/dL) have also been identified to increase bleeding risk in relation to alteplase.

The bleeding risk can be partially estimated from the plasma level of fibrinogen. Due to its relative fibrin-specificity, alteplase results in a modest decrease of the circulating plasma fibrinogen levels to about 60 % at 4 hours, which is generally reverted to more than 80 % after 24 hours. Plasminogen and alpha-2-antiplasmin decrease to about 20 % and 35 % respectively after 4 hours and increase again to more than 80 % at 24 hours [P86-5233]. A marked and prolonged decrease of the circulating fibrinogen level is only seen in few patients [P89-46753]. A plasma level of fibrinogen below 150 mg/dL has been associated with an elevated risk of bleeding [P13-13102]. The bleeding risk can be minimized by intensive monitoring of plasma fibrinogen levels and establishing stopping criteria or by dose reduction.

Vasculature health of the patient seem to play a major role in the bleeding risk. Confounding conditions affecting the vasculature health include high blood pressure, T2DM and advanced age. These conditions are considered patient-related risk factors for bleeding.

Alteplase administered intravenously carries a risk of bleeding and the extent of this risk is multifactorial (e.g. dose, weight, age, gender, blood pressure, diabetes) [P15-13160]. The highest number of bleeding events were seen in trials involving acute ischemic stroke patients, where the reported complications with alteplase total doses (up to 90 mg) given over 2 hours were symptomatic intracranial haemorrhage (6%) and other major haemorrhage (2%). Minor haemorrhage (e.g. suction through endotracheal tube or at site of venepuncture) accounting for 20-25%.

Similarly in patients with acute MI where alteplase doses (up to 100 mg) given over 2 to 3 hours were applied, the risk of intracranial haemorrhage is <1%. In multivariable models the main risk factors were older age, female sex, black ethnicity, systolic blood pressure (BP) of 140 mm Hg or above, diastolic blood pressure of 100 mm Hg or above, history of stroke, alteplase dose of more than 1.5 mg/kg, and lower body weight [P98-10462].

Another common site of bleeding is gastrointestinal (GI) bleeding, which is often not fatal and can be managed as per standard protocols. In a randomised trial of pharmaco-mechanical-thrombolysis with alteplase dosages of <35 mg, major bleeding was seen in approximately 1.7% of patients (6 of 336 treated patients) which consisted of two patients with GI bleeding, two patients with retroperitoneal bleeding and two patients with bleeding at the site of the procedure. All patients were receiving concomitant anticoagulation [[P17-13366](#)].

Hypersensitivity

Further known risks of alteplase are hypersensitivity reactions. These can occur at any time and are not predictable. If detected at an early stage, any form of hypersensitivity reaction (anaphylaxis, anaphylactic reactions like angioedema) can be effectively treated with medication such as adrenaline/epinephrine, glucocorticoids, antihistamines, complement inhibitor, bradykinin antagonists and fluids.

Effect of Alteplase on D-Dimer and fibrinogen

In patients with acute myocardial infarction (AMI) or deep vein thrombosis (DVT), D-dimer and fibrin degradation products significantly increased during administration of alteplase, while fibrinogen and plasminogen concentrations significantly decreased compared to baseline [[P90-52475](#), [P91-62566](#)]. The increase in D-dimer levels was correlated with thrombus mass and can be considered as indicator for efficient thrombolytic treatment in AMI and DVT patients [[P90-52475](#), [P92-71704](#)].

Overview of trial-related risks

An overview of trial-related risks is provided in [Table 1.4.2:1](#).

Table 1.4.2:1 Overview of trial-related risks

Possible or known risks of clinical relevance for this trial	Summary of data, rationale for the risk	Mitigation strategy
Investigational Medicinal Product / alteplase		
Cardiovascular: hypotension, bradycardia GI: nausea, vomiting, GI tract bleeding Haematologic: spontaneous bleeding, bone marrow depression Musculoskeletal: musculoskeletal pain Respiratory: Pulmonary edema Skin: bruising, flushing Other: fever, edema, phlebitis	More detailed description see IB, Section 7.6	Timely detection, evaluation, and follow-up of laboratory alterations in selected laboratory parameters to ensure patients' safety. ECG monitoring in ICU, repeated ECGs. Monitoring for bleeding including neurological assessment to rule out intracranial haemorrhage. Monitor hypersensitivity reactions such as angioedema, profound hypotension, rash, urticaria. Supportive treatment for bleeding including infusion therapy, red blood cell (RBC) and FFP transfusion. In case of hypersensitivity glucocorticoids, antihistamines, and adrenaline (epinephrine) address acute hypersensitivity. Intubation readiness
Clinical events		
Minor bleeding particularly traumatic – suction through endotracheal tube or at site of venepuncture		Supportive treatment, and use caution during suction, measurement of plasma fibrinogen levels

Possible or known risks of clinical relevance for this trial	Summary of data, rationale for the risk	Mitigation strategy
Major bleeding including intracerebral haemorrhage	Common	Measurement of plasma fibrinogen levels, recommendation to stop alteplase infusion, and administer fibrinogen concentrate or cryoprecipitate, if fibrinogen is <150 mg/dL and other supportive treatment including i.v. tranexamic acid.
Hypersensitivity reactions (e.g. rash, urticaria, bronchospasm, angio-oedema, hypotension, shock).	Rare	Anti-allergic medications
Serious anaphylaxis	Very rare	Treat per local protocols and stop further administration of medication
Other risks		
Additional blood samples	Bleeding related to venipuncture	Compression
Transfusion of blood products, fibrinogen or supportive treatment like i.v. tranexamic acid.	Related to bleeding event. Anaphylaxis due to transfusion.	Treatment as per local protocols and product information

Risk evaluation in relation to COVID-19:

Patients participating in this trial are at higher risk for severe consequences resulting from COVID-19. In the event of restriction to visit the investigative site (for patients after discharge) and in case a visit to the hospital is not possible for an individual patients, certain procedures can be done remotely, and local labs obtained from a laboratory located outside the hospital can be used instead of the hospital laboratory. These changes are meant to keep the integrity of the trial and they will not affect the benefit-risk for the individual patient.

1.4.3 Discussion

Consideration of therapies that are not recognized for this indication but of potential benefit, such as fibrinolytic agents, is warranted in this unprecedented public health emergency of COVID-19.

The bleeding risk described in the literature typically include high dosages of alteplase (up to 90 to 100 mg given as infusion over 1-3 hours) and the concurrent use of therapeutic anticoagulants. However, the alteplase short-term infusion dosages being used in this study (0.3 mg/kg/2h or 0.6 mg/kg/2h) are lower than those used in studies for MI, massive PE and stroke. Additionally, by the low dose during long-term infusion over 12 hours, the rapid inactivation of alteplase in the circulation is projected to be associated with plasma fibrinogen levels well above the threshold of 150 mg/dL, which are known to be linked to the risk bleeding [[c32479735-01](#); [P13-13102](#)].

The bleeding risk is predicted from the plasma fibrinogen level which provides a measure of the alteplase absorbed. This will be monitored and if the level falls below 150 mg/dL, the investigator is asked to evaluate whether the clinical condition of the patient warrants a temporary interruption of subsequent alteplase administration. Beside the extent of fibrinogen breakdown, D-Dimer levels, thrombocytopenia, the prolongation of the activated partial thromboplastin time (aPTT) will be monitored continuously. Furthermore, elevated blood pressure as a risk of intracranial hemorrhage and the co-administration of ACE inhibitors related to the risk of angioedema will be monitored.

Taken together, the concept of combining a moderate initial dose of alteplase applied over 2 hours followed by long-term low dose infusion to COVID-19 related patients with ARDS is considered to have a favorable benefit-risk ratio. The expected benefit is an improved pulmonary oxygenation via lysis of pulmonary vascular microthrombi and fibrin deposition in the alveoli. The risk of bleeding is considered acceptable in this severely ill population and will be monitored by measurements of fibrinogen and coagulation parameters. Precautionary recommendations for temporary interruption of alteplase infusion and concomitant dose adjustments of heparin will be provided to mitigate the risk of bleeding.

2. TRIAL OBJECTIVES AND ENDPOINTS

2.1 MAIN OBJECTIVES, PRIMARY AND SECONDARY ENDPOINTS

2.1.1 Main objectives

Main objective is to evaluate the efficacy and safety of two (Part 1) different dosing regimen and of one dosing regimen (Part 2) of intravenous alteplase given for up to 5 days on top of standard of care (SOC) compared with SOC alone in ARDS associated with COVID-19. SOC includes supportive measures, such as the use of non-invasive or invasive ventilation, haemodynamic support, if needed, sedation, as well as medical therapies commonly used in patients suffering from ARDS or its complications. SOC follows the standard therapies established locally.

2.1.2 Primary endpoint

The same primary endpoint will be evaluated in Part 1 and in Part 2, as follows:

- 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. See [Section 5.1.1](#).

2.1.3 Secondary endpoints

The same secondary endpoints to assess safety and efficacy in this setting will be evaluated in Part 1 and in Part 2, as follows:

- All cause mortality at Day 28
- Number of ventilator-free days from start of treatment to Day 28
- Improvement of Sequential (sepsis-related) Organ Failure Assessment (SOFA) score by ≥ 2 points from baseline to end of Day 6
- Major bleeding events (MBE) (according to International Society on Thrombosis and Haemostasis [ISTH] definition[[R05-0344](#)] until Day 6, see [Section 5.2.5.1](#).
-
- Daily average PaO₂/FiO₂ ratio (or inferred PaO₂/FiO₂ ratio from SpO₂) change from baseline to Day 6
- All-cause mortality or on mechanical ventilation at Day 28

2.2 FURTHER OBJECTIVES AND FURTHER ENDPOINTS

2.2.1 Further objectives

Further objectives are to evaluate additional and complimentary details on efficacy and safety, and to obtain pharmacokinetic data.

2.2.2 Further endpoints

The same further endpoints will be evaluated in Part 1 and in Part 2 as follows:

- Length of ICU stay
- Length of hospital stay
- Evolution of daily average $\text{PaO}_2/\text{FiO}_2$ (or inferred $\text{PaO}_2/\text{FiO}_2$ from SpO_2) ratio from baseline until Day 28
- Evolution of 11-point WHO Clinical Progression Scale up to Day 28, according to baseline status.
- Evolution of APACHE 2 score from baseline to Day 28
- Time to intubation (in patients not on mechanical ventilation at baseline)
- Time to successful extubation (in patients on mechanical ventilation at baseline, i.e. no re-intubation until discharge)
- Time to extracorporeal membrane oxygenation (ECMO)
- Changes in coagulation and inflammatory markers on treatment, as available from local laboratory measurements, such as D-Dimer, fibrinogen, platelets, aPTT, International normalised ratio (INR), C-reactive protein (CRP), procalcitonin, PAI-1, Interleukin-6, thrombomodulin and ferritin.
- Days free of inotropes/vasopressors up to Day 28
- Time to first major bleeding event (MBE, according to ISTH definition) until Day 28
- Time to drop of fibrinogen to <150 mg/dL
- All cause mortality at Day 90

Pharmacokinetic endpoints are as follows:

- $\text{Css},1$ (plasma concentration of alteplase at steady state of 2 hour infusion)
- $\text{Css},2$ (plasma concentration of alteplase at steady state of long-term infusion)

Further safety endpoints include the following:

- Stroke on treatment and until Day 28
- Myocardial infarction on treatment and until day 28
- Pulmonary embolism on treatment and until Day 28
- Deep vein thrombosis on treatment and until Day 28

3. DESCRIPTION OF DESIGN AND TRIAL POPULATION

3.1 OVERALL TRIAL DESIGN

This is an open-label operationally seamless Phase IIb/III randomised, sequential, parallel-group adaptive clinical trial in patients experiencing ARDS triggered by COVID-19, comparing daily intravenous infusion of alteplase, up to a maximum treatment duration of 5 days on top of SOC, versus SOC alone. This trial will investigate the efficacy and safety of different dosing regimens in hospitalized patients with ARDS of mild to moderate severity.

The study comprises two parts in an operationally seamless sequential adaptive design: Part 1 (dose-finding, “proof of concept”, Phase IIb) with 60 patients using a 1:1:1 randomisation scheme to two dose regimens of alteplase treatment on top of SOC, or SOC alone; and Part 2 (confirmatory, Phase III) with 210 patients carrying one dosing regimen forward and using a 2:1 randomisation to alteplase treatment on top of SOC, or SOC alone. The two parts include the same patient population, and have same objectives and endpoints. Visit schedules are the same in both parts. There will be one interim assessment after Part 1. One dosing regimen of alteplase will be selected for Part 2, based on the benefit-risk profile observed in Part 1.

To minimize the bias eligible patients will be randomised using Interactive Response Technology (IRT). The randomisation list will be generated using a validated system, which involves a pseudo-random number generator so that the resulting treatment will be reproducible and non-predictable. The block size will be documented in the CTR. Access to the codes will be controlled and documented.

Randomisation in Part 1 will be 1:1:1 to one of the three arms:

- initial i.v. infusion of alteplase 0.3 mg/kg/2h (Day 1) followed by daily i.v. long-term infusion of 0.02 mg/kg over 12 hours (Dosing regimen A),
- initial i.v. infusion of alteplase 0.6mg/kg/2h (Day 1) followed by daily i.v. long-term infusion of 0.04 mg/kg/h over 12 hours (Dosing regimen B)
- or SOC.

Randomization will be stratified by type of ventilation (non-invasive or invasive mechanical).

Randomisation in Part 2 will be 2:1 to one dosing regimen of alteplase and to standard of care, and will be stratified by type of ventilation (non-invasive or invasive mechanical).

This is an open-label study, therefore no blinding will apply for patients, investigators and personnel at the site involved in trial conduct. More details please see [Section 4.1.5.1](#).

A schematic overview of trial design is shown in ([Figure 3.1:1](#)).

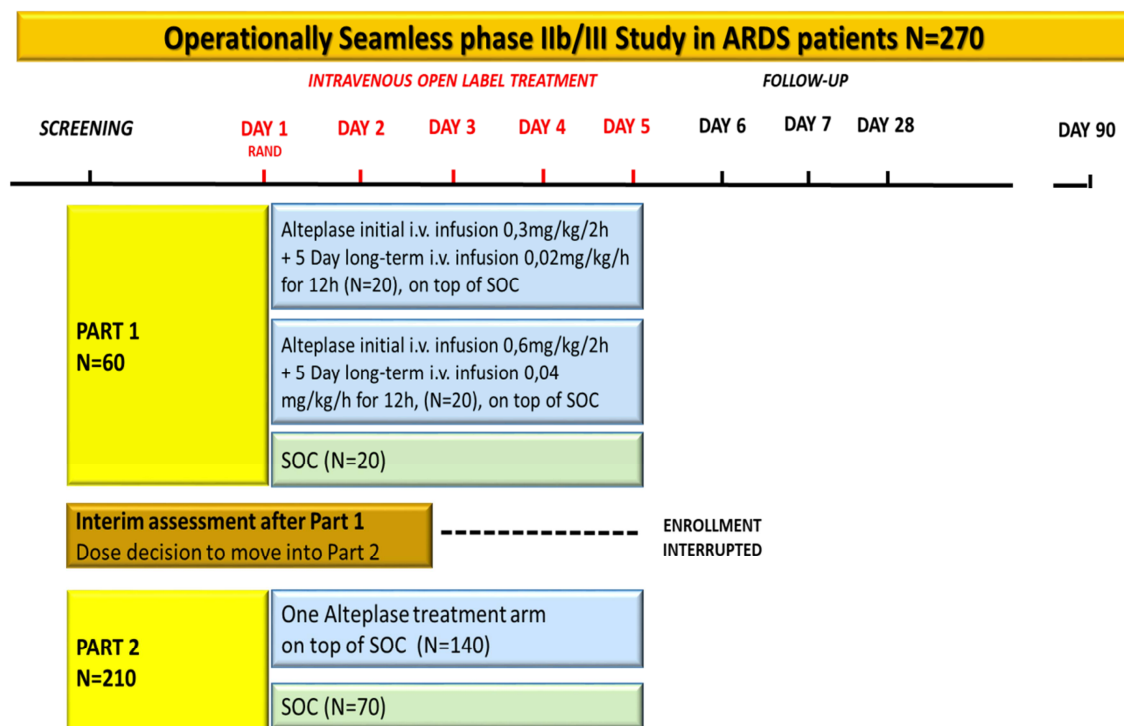


Figure 3.1:1 Study design

3.2 DISCUSSION OF TRIAL DESIGN, INCLUDING THE CHOICE OF CONTROL GROUP(S)

Operationally seamless trial design, with separate proof-of-concept part (Part 1, Phase IIb) and confirmation of efficacy and safety part (Part 2, Phase III). The trial will include pre-defined decision criteria for selecting one alteplase dosing regimen for Part 2, with the intent of ensuring that a positive proof-of-concept will quickly be followed by the confirmation of efficacy and safety of alteplase in patients with ARDS. Should Part 1 results indicate the need for design modifications in Part 2 beyond those pre-specified in the protocol, these required modifications will be implemented via a global CTP amendment.

An additional advantage of the operationally seamless adaptive design for the trial sites is mainly to gain experience with the protocol measures. The anticipated short duration between the end of Part 1 and the start of Part 2 will reduce the risk of potential measurement errors and protocol deviations in Part 2, the confirmatory part of the trial. The overall expectation is an increase in site efficiency and study quality. In addition, the Part 1 experience with protocol measures is expected to lead to a reduction in the variability associated with efficacy measurements thereby increasing the precision of treatment estimates particularly in the confirmatory part (Part 2) and to ensure that safety assessments are as objective as possible.

Finally, the accelerated recruitment compared with two separate trials may reduce time to availability of an effective treatment filling unmet medical needs in patients with ARDS.

A parallel group, sequential, randomized, open label trial was considered most appropriate to demonstrate proof of concept of alteplase in Part 1 in patients with Covid-19-induced ARDS. The SOC control group in Parts 1 and 2 is thus required to compare both efficacy and safety with the alteplase treatment arm. Sites are expected to ensure that patients are provided with best possible standard of care for the treatment of ARDS according to local guidelines.

As an established thrombolytic therapy, alteplase may offer benefit for patients with ARDS, associated with COVID-19, by targeting the coagulation and fibrinolytic systems [[R20-1760](#)]. Currently, such severely ill patients lack effective treatment besides supportive care.

In order to ensure the patient's safety during the trial, a fully external data monitoring committee (DMC), independent of the trial and project teams, will be set-up to review all available un-blinded safety data as well as selected efficacy data at regular intervals following first patient in (FPI) for Part 1 and Part 2, respectively. The role of DMC is given below, further details including pre-specified rules for decision-making, will be provided in a DMC charter.

The DMC's role is to monitor patients' safety continuously and to put it in context with observed treatment benefits. The DMC may provide recommendations to the Sponsor and the Expert Board on the following: (1) Changes in dosing (e.g. change in dose or timing of infusion schedule, changing criteria for treatment interruption, concomitant or restricted medication, potential closure of one dose group in Part 1, additional safety measures to be implemented), (2) Changes in population selection (e.g. exclude select patients based on data generated from the trial, modify in-/exclusion criteria), (3) Termination of the trial in case of serious safety concerns, (4) Stop the trial for futility, (5) Any other measure that affects patients' safety. After Part 1, the DMC will provide a recommendation to continue or not continue into Part 2, together with a recommendation on the dosing regimen and the planned number of patients. For further details, see the DMC Charter.

3.3 SELECTION OF TRIAL POPULATION

Approximately 60 patients are planned to be randomised in Part 1 at approximately 30 to 35 study sites; each site is expected to randomise approximately 2 patients. In Part 2, there are approximately 210 patients planned to be randomised at approximately 55 sites and approximately 4 patients per site are planned to be randomised per site in Part 2. The majority of sites in Part 1 are located in 10 countries in Western Europe, but if recruitment numbers are not achieved, the trial may expand to Eastern Europe, Russia, China, and South America to focus on places where the incidence of COVID-19 is high. Approximately 14 countries are planned for Part 2. Given the expected regional fluctuation in SARS-CoV-2 infection rates, it may become necessary to open new countries and sites and to potentially suspend existing sites, or countries.

Screening of patients for this trial is competitive, i.e. screening for the trial will stop at all sites at the same time once a sufficient number of patients has been screened. Investigators

will be notified about screening completion and will then not be allowed to screen additional patients for this trial. This applies to both parts of the study.

A log of all patients enrolled into the trial (i.e. who have signed informed consent) will be maintained in the Investigator Site File (ISF) irrespective of whether they have been treated with investigational drug or not.

If a patient is enrolled in error (does not meet all inclusion criteria or meets one or more exclusion criteria on the day of enrolment), the sponsor should be contacted immediately.

Patients will be selected for the trial according to pre-defined eligibility criteria and written informed consent has to be obtained prior to initiation of any trial related procedures.

Randomisation of patients shall happen no later than 24 hours after the confirmation of the eligibility criteria, and study drug administration will be started within 6 hours of the randomisation.

The respiratory status of the enrolled patients is monitored by $\text{PaO}_2/\text{FiO}_2$ ratio (or inferred $\text{PaO}_2/\text{FiO}_2$ ratio from SpO_2) and should be measured at least three times per day in all patients together with a recording of the patient's position, and all available data points will be collected until discharge, death, or Day 28, whichever occurs first. The very first and the last $\text{PaO}_2/\text{FiO}_2$ ratio (or inferred $\text{PaO}_2/\text{FiO}_2$ ratio from SpO_2) should preferably be obtained, when the patient is in a supine position.

The end of trial for an enrolled patient is the date of death or Day 28, whichever occurs first.

3.3.1 Main diagnosis for trial entry

Please refer to [Section 8.3.1](#) for the documentation requirements pertaining to the eligibility criteria.

In both parts, Part 1 (proof of concept) and Part 2 (confirmatory), the trial will include patients with ARDS due to COVID-19 infection.

3.3.2 Inclusion criteria

Eligibility criteria for Parts 1 and 2 are the same. Patients participating in Part 1 are not eligible for Part 2.

1. Age ≥ 18 years (or above legal age, e.g. UK ≥ 16 years)
2. ARDS with $\text{PaO}_2^*/\text{FiO}_2$ ratio >100 and ≤ 300 , either on non-invasive ventilator support, OR on mechanical ventilation (<48 hours since intubation),
 - with bilateral opacities in chest X-ray or CT scan (not fully explained by effusions, lobar/lung collapse, or nodules)
 - with respiratory failure (not fully explained by cardiac failure/fluid overload)

*or estimation of PaO₂/FiO₂ from pulse oximetry (SpO₂/FiO₂) (see [Appendix 10.3](#) for SpO₂/FiO₂ estimation)

3. SARS-CoV-2 positive (laboratory-confirmed RT PCR test)
4. Fibrinogen level \geq upper limit of normal (according to local laboratory)
5. D-Dimer \geq 3-fold of upper limit of normal (ULN) according to local laboratory
6. Signed and dated written informed consent in accordance with ICH Good Clinical Practice (GCP) and local legislation prior to admission to the trial.

3.3.3 Exclusion criteria

1. Massive confirmed PE with haemodynamic instability at trial entry
2. Patients on mechanical ventilation for longer than 48 hours
3. Chronic pulmonary disease i.e. with known FEV₁ <50%, requiring home oxygen, or oral steroid therapy or hospitalisation for exacerbation within 12 months, or significant chronic pulmonary disease in the Investigator's opinion, or primary pulmonary arterial hypertension
4. Has a Do-Not-Intubate (DNI) or Do-Not-Resuscitate (DNR) order
5. In the opinion of the investigator not expected to survive for > 48 hours after admission
6. Patients with known hypersensitivity to the active substance alteplase, gentamicin (a trace residue from the manufacturing process) or to any of the excipients
7. Significant bleeding disorder at present or within the past 3 months, known haemorrhagic diathesis
8. Patients receiving effective oral anticoagulant treatment, e.g. vitamin K antagonists with INR >1.3, or any direct oral anticoagulant within the past 48 hours
9. Any history of central nervous system damage (i.e. neoplasm, aneurysm, intracranial or spinal surgery)
10. History or evidence or suspicion of intracranial haemorrhage including sub-arachnoid haemorrhage
11. Severe uncontrolled arterial hypertension (according to the investigator's judgement)
12. Major surgery or significant trauma in the past 10 days, recent trauma to head or cranium
13. Cardiac arrest and/or cardiopulmonary resuscitation during the current hospital stay
14. Obstetrical delivery within the past 10 days
15. Severe hepatic dysfunction, including biopsy confirmed hepatic cirrhosis, portal hypertension, hepatic encephalopathy, or active hepatitis
16. Bacterial endocarditis, pericarditis
17. Acute pancreatitis
18. Documented ulcerative gastro-intestinal disease during the last 3 months
19. Severe heart failure (New York Heart Association Class IV)
20. Arterial aneurysms, arterial/venous malformations
21. Malignancy (Stage IV)
22. Haemorrhagic stroke or stroke of unknown origin at any time
23. Ischaemic stroke or transient ischaemic attack (TIA) in the preceding 6 months
24. Patients who must or wish to continue the intake of restricted medications (see [Section 4.2.2.1](#)) or any drug considered likely to interfere with the safe conduct of the trial. Re-screening is allowed once within the same hospital stay.

25. Women who are pregnant. (Note that women of childbearing potential are not excluded as eligible patients are severely ill during the observation period of the trial.)
26. End stage renal disease (Stage ≥ 4) or need for renal replacement therapy at baseline
27. Need for ECMO at baseline
28. Platelet count $<100 \times 10^9/L$ or history of Heparin-Induced Thrombocytopenia
29. Lumbar puncture within past 3 days
30. Known abdominal or thoracic aneurysm
31. Active tuberculosis

3.3.4 Withdrawal of patients from treatment or assessments

Patients may discontinue trial treatment or withdraw consent to trial participation as a whole (“withdrawal of consent”) with very different implications; please see Sections 3.3.4.1 and [3.3.4.2](#) below.

Every effort should be made to keep the patients in the trial: if possible on treatment, or at least to collect important trial data (e.g. data required for primary and secondary outcome measures or at least vital status at planned EOS for the patient).

Measures to control the withdrawal rate include careful patient selection, appropriate explanation of the trial requirements and procedures prior to trial enrolment, as well as the explanation of the consequences of withdrawal.

The decision to discontinue trial treatment or withdraw consent to trial participation and the reason must be documented in the patient files and eCRF. If applicable, consider the requirements for Adverse Event collection reporting (please see [Sections 5.2.6.2.1](#) and [5.2.6.2.2](#)).

3.3.4.1 Discontinuation of trial treatment

An individual patient will discontinue trial treatment if:

- The patient wants to discontinue trial treatment, without the need to justify the decision.
- The patient needs to take a concomitant medication that interferes with the investigational medicinal product (IMP) or other trial treatment. Please refer to [Section 4.2.2](#) for restricted medications during this trial.
- The patient can no longer receive trial treatment for medical reasons, such as surgery, adverse events, etc. including:
 - safety reasons, such as active bleeding, or any other condition that is considered a safety risk at the discretion of investigator
 - in case of clinical improvement of respiratory situation with sustained (as per investigator's judgement) PaO_2/FiO_2 ratio above 300.
 - The alteplase long-term i.v. infusion should be discontinued, if the patient requires ECMO or RRT, and no additional dose of 0.3 mg/kg/2 h or 0.6 mg/kg/2 h should be given under these circumstances

Recommendation for temporary interruption of alteplase daily infusions:

- All catheters and access systems should preferably be inserted before the start of the treatment period.
- Interventions during the treatment period with alteplase should be avoided, whenever possible.

In case of an unavoidable intervention:

- To decrease the risk of bleeding events, invasive interventions such as pleural drainage or catheter placement should preferably be performed *during the 12 h treatment breaks*. Complete haemostasis (which can take several hours to be achieved) is required before re-starting alteplase.
- Alteplase infusion may be temporary interrupted if the fibrinogen level decreases to levels <150 mg/dL. The infusion should be restarted when fibrinogen recovers, as per investigator's judgment.

Management of Bleeding during Alteplase Treatment:

Should serious bleeding occur, in particular cerebral or pulmonary haemorrhage, the fibrinolytic therapy must be discontinued and concomitant heparin administration should be terminated immediately. Administration of protamine should be considered if unfractionated heparin has been administered. In the few patients who fail to respond to these conservative measures, judicious use of transfusion products may be indicated. Transfusion of cryoprecipitate, fresh frozen plasma, and platelets should be considered with clinical and laboratory reassessment after each administration. A target fibrinogen level of 1g/L is desirable with cryoprecipitate infusion. Antifibrinolytic agents should also be considered.

If new efficacy/safety information becomes available, BI will review the benefit-risk assessment and, if needed, pause or discontinue the trial treatment for all patients or take any other appropriate action to guarantee the safety of the trial patients.

Even if the trial treatment is discontinued, the patients remain in the trial and, given their agreement, will undergo the procedures for early treatment discontinuation and follow-up until Visit 6/EOS as outlined in the [Flow chart](#) and [Section 6.2.3](#).

3.3.4.2 Withdrawal of consent to trial participation

Patients may withdraw their consent to trial participation at any time without the need to justify the decision.

If a patient wants to withdraw consent, the investigator should be involved in the discussion with the patient and explain the difference between trial treatment discontinuation and withdrawal of consent to trial participation, as well as explain the options for continued follow-up after trial treatment discontinuation, please see [Section 3.3.4.1](#) above.

For patients who discontinue treatment prematurely, vital status shall be collected at planned completion date of each individual patient.

3.3.4.3 Discontinuation of the trial by the sponsor

Boehringer Ingelheim reserves the right to discontinue the trial overall or at a particular trial site at any time for the following reasons:

1. Failure to meet expected enrolment goals overall or at a particular trial site.
2. New efficacy or safety information invalidating the earlier positive benefit-risk-assessment, please see [Section 3.3.4.1](#).
3. Deviations from GCP, the trial protocol, or the contract impairing the appropriate conduct of the trial.

Further follow up of patients affected will occur as described in Section 3.3.4.1.

The investigator / the trial site will be reimbursed for reasonable expenses incurred in case of trial termination (except in case of the third reason).

4. TREATMENTS

4.1 INVESTIGATIONAL TREATMENTS

4.1.1 Identity of the Investigational Medicinal Products

Table 4.1.1:1 Test product

Substance:	Alteplase (recombinant tissue plasminogen activator)
Pharmaceutical formulation:	Powder and solvent for solution for injection/infusion
Source:	Boehringer Ingelheim Pharma GmbH & Co. KG
Unit strength:	50 mg/vial powder and 50 mL solvent
Posology:	<p>Initial i.v. infusion (Day 1 only):</p> <p>Part 1:</p> <ul style="list-style-type: none"> Dosing regimen A: 0,3 mg/kg given over 2 hours Dosing regimen B: 0,6 mg/kg given over 2 hours <p>Alteplase 50 mg powder for solution for injection/infusion will be reconstituted in 50 mL water for injections to yield a concentration of 1 mg/mL (= stock solution). A Perfusor Syringe of 50 mL should be used.</p> <p><u>Dosing regimen A, Initial i.v.infusion (0.3 mg/kg to be infused over 2 h)</u></p> <p><u>Dosing regimen B, Initial i.v. infusion (0.6 mg/kg to be infused over 2 h) .</u></p> <p>Part 2: One dosing regimen will be carried forward based on results from Part 1 and recommendation by the DMC for Part 2.</p>
	<p>Long-Term i.v. infusion for 12 hours per day, from Day 1 onwards starting right after the initial 2h i.v. infusion, followed by an approximately 12 hour break. This infusion scheme (12 hour infusion, followed by an</p>

	<p>approximately 12 hour break) is repeated daily up to at maximum Day 5). Preferably, the infusion will be started at approximately the same time at each day.</p> <p>Please note: On Day 1, the total infusion time is 2 hours plus 12 hours = 14 hours.</p> <p>Please note: If the 12 hour infusion has to be interrupted due to e.g. an intervention, the remaining infusion time for this day should be completed after the intervention in order to sum up to a total of 12 hours of long-term infusion on that day. The break thereafter should not be shortened to less than 12 hours.</p> <p>If the investigator decides to temporarily interrupt the 12 hour infusion due to a drop of fibrinogen level < 150 mg/dL, it is up to the discretion of the investigator to restart the infusion at any time until Day 5. However, missed days of long-term infusion will not be added to the total infusion time window, which is until at maximum Day 5 after treatment start.</p> <p>Alteplase 50 mg powder for solution for injection/ infusion will be reconstituted in 50 mL water for injections to yield a concentration of 1 mg/mL (=stock solution).</p> <p>A Perfusor Syringe of 50 mL should be used.</p> <p>A new vial of alteplase should be taken every day.</p> <p>Dose of Long-Term i.v. infusion in Part 1:</p> <p>Dosing regimen A: 0.02 mg/kg/hour given over 12 hours</p> <p>Dosing regimen B: 0.04 mg/kg /hour given over 12 hours</p> <p>Dose of Long-Term i.v. infusion in Part 2: One dosing regimen will be carried forward based on results from Part 1 and recommendation by the DMC</p>
	<p>Optional additional i.v. infusion (allowed one time, during Days 2 to 5):</p> <p>Part 1: One optional additional i.v. infusion:</p> <p>Dosing regimen A : 0.3 mg/kg given over 2 hours</p>

	<p>Dosing regimen B: 0.6 mg/kg given over 2 hours This additional i.v. infusion can be given one time during Days 2 to 5 in case of clinical worsening (as per investigator judgement)</p> <p>Please note: This optional single additional infusion of alteplase may only be given <u>one time</u>. This additional dose may be administered on <u>either</u> Day 2, 3, 4, or 5. The duration of the long-term infusion (0.02mg/kg/h over 12 hours in Dosing regimen A; 0.04mg/kg/h over 12 hours in Dosing regimen B) on the day of optional additional infusion should sum up to 12 hours. The long-term-infusion should not be given in parallel to the optional additional dose, but should be interrupted for approximately 2 hours, when the optional additional infusion of 0.3mg/kg/2h or 0.6mg/kg/2h is given. On the day of optional additional infusion, the total infusion time is 2 hours plus 12 hours = 14 hours.</p> <p>For the optional additional i.v. infusion application, it is recommended that fibrinogen concentrations are \geq 300mg/dL.</p> <p>Part 2: One dosing regimen will be carried forward based on results from Part 1 and recommendation by the DMC</p>
	<p>For Instructions for Preparation and Administration of Trial Medication, please see ISF. For dose calculation and infusion rates, please see Appendix 10.5.</p>
Mode of administration:	Intravenous infusion

4.1.2 Selection of doses in the trial and dose modifications

Selection of doses in the study took into consideration the established safety profile of alteplase and was in addition based on pharmacokinetic and pharmacodynamics (PK-PD) modelling results [[c32479735-01](#)]. Doses for Part 1 were chosen to be lower than in the current indication of alteplase for massive, haemodynamic relevant PE, because COVID-19 is associated with a constant pro-inflammatory trigger of coagulation activation, leading primarily to fibrin deposition, micro-clots and non-massive PE. Dose selection was based on the fact that the fibrinogen level should not fall below 4.4 μ M (150 mg/dL), which has been

reported as threshold for increased risk of bleeding [[P13-13102](#)]. With both dosing regimens (A and B) the fibrinogen level is likely to remain above this threshold. Both dosing regimens are considered to deliver benefits for the treatment of the disease with an acceptable safety profile. The dose -response relationship and benefit-risk ratio of each dosing regimen will be evaluated in Part 1, and a decision will be taken after Part 1 to carry one dosing regimen into Part 2.

For dosing regimen A and B see [Table 4.1.1:1](#) above.

Dose modifications are not foreseen unless recommended by the DMC. One optional additional i.v. infusion of 0.3 mg/kg infused over 2 hours (Dosing regimen A) or 0.6 mg/kg infused over 2 hours (Dosing regimen B) can be given once on Days 2 to 5 in case of clinical worsening (as per investigator judgement).

If the fibrinogen decreases to levels <150 mg/dL, the investigator may stop the alteplase infusion temporarily [[c32479735-01](#); [P13-13102](#)]. The alteplase infusion can be restarted, if fibrinogen recovers, as per investigator's judgement.

4.1.3 Method of assigning patients to treatment arms

After the assessment of all eligibility criteria, each eligible patient will be randomised to treatment arms according to a randomisation plan in a 1:1:1 ratio at Visit 2a (same day as screening or day afterwards) in Part 1, and in a 2:1 randomisation rate in Part 2 via Interactive Response Technology (IRT). The appropriate medication number will be assigned and documented in the eCRF.

The randomization will be stratified by the type of ventilation support (invasive mechanical / non-invasive) and access to the randomization code will be controlled and documented. The block sizes of the randomization will be documented in the clinical trial report (CTR) and patients will be randomized sequentially.

Note that the medication number is different from the patient number (the latter is generated during screening via the IRT System).

4.1.4 Drug assignment and administration of doses for each patient

At Day 1, patients will be randomized to one of three treatment arms (1:1:1) in Part 1 or to one of two treatment arms (2:1) in Part 2 by the IRT.

9 vials will be allocated per each patient in higher dose arm and 7 vials will be allocated per each patient in lower dose arm. In case additional kits are needed reflecting calculated amount needed per body weight, site has to call IRT and register a replacement call to allocate the additionally needed kits.

Each patient kit contains:

1 vial alteplase (TPA-05) Powder for solution for injection/infusion (50 mg/vial) with 50 mL solvent for alteplase (TPA-05) powder in a box. All components are labelled in an open label fashion. Single panel labels are placed on vials and box.

The pharmacist or his/her qualified delegate will prepare the trial medication at the dose level requested by the investigator. For dose calculation and infusion rates please refer to Alteplase dosing table in [Appendix 10.5](#).

Handling and preparation of the medicinal product should be performed according to routine site procedures using aseptic techniques. Prior to the reconstitution, preparation and the infusion, the vials should be checked for any changes (e.g. color) and for the absence of particles. The lyo cake should be a white to pale yellow cake, the solution should be clear and colorless to slightly yellow. If you have any questions or concerns regarding appearance of the solution, please contact your CRA or the local Clinical Monitor.

The reconstitution should be done immediately before use.

Reconstitution can be performed with a transfer cannula (if available in the kit) or with syringe and cannula. More details on preparation, administration and in-use stability of alteplase solution will be provided in Instructions for Preparation and Administration of Alteplase 50 mg for solution for injection/infusion in ISF.

Table 4.1.4:1 Dosage and treatment schedule (Part 1)

Initial i.v. infusion (Day 1):

- Dosing regimen A: 0.3 mg/kg infused over 2 hours
- Dosing regimen B: 0.6 mg/kg infused over 2 hours

Daily long-term i.v. infusion over 12 hours from Day 1 onwards, up to at maximum Day 5:

	Dosing regimen A	Dosing regimen B
Target Dose	0.02 mg/kg/h	0.04 mg/kg/h

Optional additional i.v. infusion of 0.3 mg/kg infused over 2 hours (Dosing Regimen A) or 0.6 mg/kg infused over 2 hours (Dosing regimen B) is allowed once, on Days 2 to 5.

Part 2: One dosing regimen will be carried forward based on results from Part 1 and recommendation by the DMC.

4.1.5 Blinding and procedures for unblinding

4.1.5.1 Blinding

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 will be kept restricted until it is released for analysis. The randomisation codes will be provided to bioanalytics prior to last patient completed in each part of the trial to allow for a timely analyses of pharmacokinetic (PK) samples.

4.1.5.2 Unblinding and breaking the code

As this is an open-label trial, unblinding does not apply for the investigator and study site personnel.

Due to the requirements to report Suspected Unexpected Serious Adverse Reactions (SUSARs), it may be necessary for a representative from BI's Pharmacovigilance (PV) group to access the randomisation code for individual patients during trial conduct. The access to the code will only be given to authorised PV representatives for processing in the PV database system and not be shared further.

4.1.6 Packaging, labeling, and re-supply

The IMPs will be provided by BI or a designated Contract Research Organisation (CRO). They will be packaged and labelled in accordance with the principles of Good Manufacturing Practice. Re-supply to the sites will be managed via an IRT system, which will also monitor expiry dates of supplies available at the sites.

For details of packaging and the description of the label, refer to the ISF.

4.1.7 Storage conditions

Drug supplies will be kept in their original packaging and in a secure limited access storage area according to the recommended storage conditions on the medication label. A temperature log must be maintained for documentation. .

If the storage conditions are found to be outside the specified range, the Clinical Research Associate (CRA), as provided in the list of contacts, must be contacted immediately.

4.1.8 Drug accountability

The investigator or designee will receive the investigational drugs delivered by the sponsor when the following requirements are fulfilled:

- Approval of the clinical trial protocol (CTP) by the Institutional Review Board (IRB) / Ethics Committee (EC),
- Availability of a signed and dated clinical trial contract between the sponsor and the investigational site,
- Approval/notification of the regulatory authority, e.g. Competent Authority (CA),

- Availability of the curriculum vitae of the Principal Investigator,
- Availability of a signed and dated CTP,
- Availability of the proof of a medical license for the Principal Investigator,

Investigational drugs are not allowed to be used outside the context of this protocol. They must not be forwarded to other investigators or clinics.

The investigator or designee must maintain records of the product's delivery to the trial site, the inventory at the site, the use by each patient, and the return to the sponsor or warehouse / drug distribution centre or alternative disposal of unused products. If applicable, the sponsor or warehouse / drug distribution centre will maintain records of the disposal.

These records will include dates, quantities, batch / serial numbers, expiry ('use- by') dates, and the unique code numbers assigned to the IMP and trial patients. The investigator or designee will maintain records that document adequately that the patients were provided the doses specified by this CTP and reconcile all IMPs received from the sponsor. At the time of return to the sponsor or appointed CRO, the investigator or designee must verify that no remaining supplies are in the investigator's possession.

4.2 OTHER TREATMENTS, EMERGENCY PROCEDURES, RESTRICTIONS

4.2.1 Other treatments and emergency procedures

Anticoagulant medications should be given to prevent the formation of new clots, as follows:

Alteplase arm, Day 1 to 5 (treatment period):

The investigator may choose between Option A or B:

For exception see below.*

Option A: low-dose LMWH (e.g. enoxaparin 1 mg/kg body weight once daily or equivalent dose of other LMWH), given every day at the end of long-term i.v. alteplase infusion. The target anti-Xa level should be <0.7 (measured at trough, i.e. just before the next injection is due).

or:

Option B: Unfractionated heparin (UFH, e.g. 10 IU/ kg body weight/hour) concomitantly to long-term alteplase i.v. infusion, to be started after immediately after initial 2 hour alteplase i.v. infusion, with a target aPTT of 1.0 to 1.5 fold of ULN according to local laboratory [[U99-0193](#), [P99-02520](#)].

It is recommended to administer antithrombin III to patients where no further anticoagulant effects with dose increases of heparin are seen.

Alteplase arm, post treatment period (Day 5 and onwards), and SOC arm:

Option A: LMWH with target anti-Xa level according to local practice

or

Option B: Unfractionated heparin with target aPTT level according to local practice

**Exception: Option B is mandatory, if estimated glomerular filtration rate (GFR) is <30 mL/min/1.73m² (renal impairment)*

Heparin should not be given in parallel to the initial 2h i.v. infusion of alteplase, at time of an *optional* additional 2h i.v. infusion of alteplase, or when an intervention needs to be performed. An additional 2h i.v. infusion of alteplase should preferably be given after UFH has been stopped for 2 hours, or ideally 12 hours after the last LMWH injection, however, these interruptions intervals may not apply in an emergency situation (according to the investigator's judgement).

The interruption intervals for concomitant heparin treatment before an intervention should follow local practice. Please see product information of respective heparins for appropriate interruption intervals before intervention (for temporary interruption of alteplase, please see [Section 3.3.4.1](#)).

The anti Xa level should be monitored regularly, at least daily, the aPTT level should be monitored preferably more frequently, while given concomitantly with alteplase.

Occurrence of heparin-induced thrombocytopenia (HIT) must result in cessation of UFH or LMWH without recommencement regardless of treatment assignment. Use of an acceptable alternative agent is required in this instance as clinically indicated. Occurrence of HIT has to be reported as adverse event (AE).

Standard of Care (SOC) arm

SOC includes any supportive measures applied in hospital, specifically on an ICU, such as for example the use of non-invasive or invasive ventilation, oxygen masks, haemodynamic support, if needed, sedation, as well as medical therapies commonly used in patients suffering from ARDS or its complications. SOC should include best possible treatment regimen established locally and should be in line with current guidelines for ARDS treatment.

Emergency procedures

Beyond patient-specific risk factors for bleeding, the bleeding risk can be estimated by the plasma fibrinogen level. Fibrinogen will be monitored daily during the treatment period with alteplase, and if the level falls below 150 mg/dL, the investigator is asked to evaluate whether the clinical condition of the patient warrants a temporary interruption of subsequent alteplase administration.

In case of bleeding, intervention or surgery, the treatment with alteplase may require a temporary interruption or permanent discontinuation of alteplase, according to the investigator's discretion and the severity of the bleed. While a major bleeding may in general require a temporary interruption or premature discontinuation of alteplase, minor bleeds may

not lead to changes in study drug application. For details on temporary interruption of alteplase, please see [Section 3.3.4.1](#).

4.2.2 Restrictions

4.2.2.1 Restrictions regarding concomitant treatment

The following medications are not allowed to be used concomitantly during the treatment period in the alteplase arm:

- Vitamin K antagonists*
- Direct oral anticoagulants**
- Antiplatelet agents (with the exception of acetyl salicylic acid, ASA, up to 100 mg/day)
- GPIIb/IIIa antagonists
- Other fibrinolytic drugs (for ultima ratio/bail-out situation, see below).
- Any medication that may increase the risk of bleeding, except LMWH/unfractionated heparin as described in [Section 4.2.1](#)

The above mentioned restrictions do not longer apply after the completion of the treatment period in the Alteplase arm.

**Patients who previously received a vitamin K antagonist can only be randomised, if INR is <1.3.*

***Patients who previously received a direct oral anticoagulant, can only be included, if the direct oral anticoagulant has been stopped for at least 48 hours*

Except for the use of fibrinolytic drugs, which should not be given in the SOC arm, these restrictions do not apply for patients in the SOC arm, these patients should be treated according to institutional guidelines.

Ultima ratio situation - Bail-out:

Preferably, patients should stay on initially assigned treatment for the first 5 days, and marketed Actilyse® should not be given during the treatment period. However, if a patient fulfills an indication according to the local label for Actilyse® (e.g. ischemic stroke, massive haemodynamic instable PE, MI), marketed Actilyse® (non-study drug) is allowed in study treatment arms and SOC arms as *ultima ratio*, at the discretion of investigator at any time during the trial.

4.2.2.2 Restrictions on diet and life style

Not applicable.

4.2.2.3 Contraception requirements

No contraception requirements are applicable, as patients are hospitalized and in general are severely ill during the whole observational period.

4.3 TREATMENT COMPLIANCE

As all study treatments will be given intravenously in-hospital, no compliance problems are foreseen. The only reasons for deviations from the treatment schedule in the protocol would be due to the rare events of dosing mistakes or damaged treatment kits. Information on dosing will be collected in the eCRF.

5. ASSESSMENTS

5.1 ASSESSMENT OF EFFICACY

5.1.1 WHO Clinical Progression Scale

The following 11-point WHO Clinical Progression Scale will be used for the assessment of clinical improvement [[R20-2002](#)] ([Table 5.1.1: 1](#)):

Table 5.1.1:1 WHO Clinical Progression Scale

Patient State	Descriptor	Score
Uninfected	Uninfected ; no viral RNA detected	0
Ambulatory mild disease	Asymptomatic ; viral RNA detected	1
	Symptomatic ; independent	2
	Symptomatic ; assistance needed	3
Hospitalised : moderate disease	Hospitalised ; no oxygen therapy*	4
	Hospitalised ; oxygen by mask or nasal prongs	5
Hospitalised : severe diseases	Hospitalised ; oxygen by NIV or high flow	6
	Intubation and mechanical ventilation, $pO_2/FiO_2 \geq 150$ or $SpO_2/FiO_2 \geq 200$	7
	Mechanical ventilation $pO_2/FiO_2 < 150$ ($SpO_2/FiO_2 < 200$) or vasopressors	8
	Mechanical ventilation $pO_2/FiO_2 < 200$ and vasopressors, dialysis, or ECMO	9
Dead	Dead	10

Source: Lancet Infect Dis. 2020, published online June12, 2020 [[R20-2002](#)]

5.1.2 Ventilator-free days, days in ICU

Ventilator-free days until Day 28 will be captured from source documents. Time-points of intubation and extubation will be recorded in the eCRF. Days on ICU, intermediate care, step-down unit (normal ward) will be counted until Day 28 based on source documents. Date of discharge from hospital and/or death will be recorded in the eCRF.

5.1.3 PaO₂/FiO₂ ratio

The respiratory status of the enrolled patients is monitored by the PaO₂/FiO₂ ratio (or inferred PaO₂/FiO₂ ratio from SpO₂, see [Appendix 10.3](#)) regularly and all available data points will be collected until discharge, death, or Day 28, whatever occurs first.

The very first and the last PaO₂/FiO₂ ratio should preferably be obtained, when the patient is in a supine position. During the first 6 days and during stay on ICU (whatever is longer) PaO₂/FiO₂ ratio should be measured three times per day in all patients, together with a recording of the patient's position. Thereafter, less frequent measurements are acceptable and should be obtained according to local clinical practice. The average daily measurements for each patient will be calculated and later analysed.

5.1.4 SOFA Score

Change in Sequential (sepsis-related) Organ Failure Assessment (SOFA) score is assessed during hospital stay from baseline to end of Day 6 ([Appendix 10.2](#))

5.1.5 APACHE II Score

Change in APACHE II (Acute Physiology and Chronic Health Disease Classification System II) score is assessed from baseline until Day 28, as long as the patient is still in hospital ([Appendix 10.2](#)).

5.2 ASSESSMENT OF SAFETY

5.2.1 Physical examination

A physical examination will be performed at the time points specified in the [Flow chart](#). It includes at minimum the assessment of general appearance, neck, lungs, cardiovascular system, abdomen, extremities, and skin.

Measurement of body weight and height will be performed at the time points specified in the [Flow chart](#). The results must be included in the source documents available at the site.

5.2.2 Vital signs

Vital signs will be evaluated at the time points specified in the [Flow chart](#).

This includes systolic and diastolic blood pressure, body temperature, respiratory rate and pulse rate (electronically or by palpation count for 1 minute) after 5 minutes of rest. The results must be included in the source documents available at the site.

5.2.3 Safety laboratory parameters

Safety laboratory parameters to be assessed are listed in [Table 5.2.3:1](#). For the sampling time points please see the [Flow chart](#). Local laboratory results will be used throughout the trial.

All analyses will be performed by the local laboratory, the respective reference ranges will be provided in the ISF. Mandatory local laboratory parameters are: PaO₂, haematology, ALT, AST, creatinine, potassium, sodium, LDH, CRP, fibrinogen, D-Dimer, and either activated partial thromboplastin time (aPTT) or anti-Xa levels (depending on anticoagulant treatment regimen).

Patients do not have to be fasted for the blood sampling for the safety laboratory.

Table 5.2.3:1 Safety laboratory tests

Haematology
<ul style="list-style-type: none"> • Haematocrit • Haemoglobin • RBCs / Erythrocytes • White Blood Cells (WBC) / Leukocytes • Platelet Count / Thrombocytes
Clinical chemistry
<ul style="list-style-type: none"> • Albumin* • Alkaline phosphatase* • ALT (alanine transaminase, serum glutamic-pyruvic transaminase [SGPT]) • AST (aspartate transaminase, serum glutamic-oxaloacetic transaminase [SGOT]) • Bilirubin total* • Creatinine (<i>estimated GFR to be calculated according to local practice</i>) • Creatine kinase (CK) * • Troponin I or T* • Lactate dehydrogenase • Glucose* • Potassium • Sodium • Urea (BUN) * • Type B natriuretic peptide (BNP), or NT-proBNP*
Coagulation and Inflammation
<ul style="list-style-type: none"> • aPTT (Activated partial thromboplastin time; in patients receiving UFH) • Prothrombin time, (or alternatively INR) • D-Dimer • Fibrinogen • Antithrombin* • Anti-Xa (in patients receiving LMWH) • C-reactive Protein • Procalcitonin* • Interleukin-6* • Plasminogen activator inhibitor 1 * • Thrombomodulin* • Ferritin*

*If available locally

Clinically relevant abnormal findings as judged by the investigator will be reported as adverse events (please refer to [Section 5.2.6.1](#)).

5.2.4 Electrocardiogram

The 12-lead ECGs must be administered by a qualified personnel and results will be recorded as scheduled in the [Flow chart](#). The investigator or a designee will evaluate whether the ECG is normal or abnormal and assess clinical relevance. ECGs may be repeated for quality reasons and a repeated recording may be used for evaluation. In addition to the scheduled ECGs, ECGs should be done and documented at any time of clinical event (e.g. acute event of arrhythmia, tachy- or bradycardia, angina, or MI).

Dated and signed printouts of ECG with findings should be documented in the patient's medical records.

Clinically relevant abnormal findings will be reported either as a baseline condition (if identified at the Screening Visit) or otherwise as adverse events and will be followed up and/or treated as medically appropriate.

5.2.5 Other safety parameters

5.2.5.1 Bleeding events

Patients should be carefully assessed for signs and symptoms of bleeding. Bleeding definitions are provided below. The location of the bleeding including the specific critical area or organ into which the bleeding occurred (e.g. GI, intracranial, pulmonary bleed) and whether or not it prolongs hospitalization will be recorded.

Definition of any bleed

This is the sum of all major and minor bleeds.

Definition of a major bleed

Major bleeds will be defined according to the ISTH definition of a major bleed, as follows ([R05-0344](#)):

- Symptomatic bleeding in a critical area or organ, such as intracranial, intraspinal, intraocular, retroperitoneal, intra-articular or pericardial, or intramuscular with compartment syndrome,

and/or

- Bleeding associated with a reduction in hemoglobin of at least 2g/dL (1.24 mmol/L), or leading to transfusion of two or more units of blood or packed cells¹

and/or

¹ Bleeding should be overt and the hemoglobin drop should be considered to be due to and temporally related to the bleeding event.

- Fatal bleed

Definition of a fatal bleed

Fatal bleeding is defined as a bleeding event that the investigator determines is the primary cause of death or contributes directly to death.

Definition of intracranial hemorrhage

Intracranial hemorrhage comprises the subtypes of intracerebral bleeds, subdural bleeds, epidural bleeds and subarachnoid bleed and will be recorded in eCRF.

Definition of a minor bleed

Minor bleeds are clinical bleeds that do not fulfill the criteria for major bleeds.

5.2.6 Assessment of adverse events

5.2.6.1 Definitions of adverse events

5.2.6.1.1 Adverse event

An adverse event is defined as any untoward medical occurrence in a patient or clinical investigation patient administered a medicinal product and which does not necessarily have to have a causal relationship with this treatment.

An adverse event can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

The following should also be recorded as an adverse event in the eCRF and BI SAE form (if applicable):

- Worsening of the underlying disease or of other pre-existing conditions
- Changes in vital signs, ECG, physical examination and laboratory test results, if they are judged clinically relevant by the investigator.

If such abnormalities already exist prior to trial inclusion, they will be considered as baseline conditions and should be collected in the eCRF only.

5.2.6.1.2 Serious adverse event

A serious adverse event (SAE) is defined as any adverse event, which fulfills at least one of the following criteria:

- results in death,
- is life-threatening, which refers to an event in which the patient was at risk of death at the time of the event; it does not refer to an event that hypothetically might have caused death if more severe,
- requires inpatient hospitalisation or prolongation of existing hospitalisation
- results in persistent or significant disability or incapacity,

- is a congenital anomaly / birth defect,
- is deemed serious for any other reason if it is an important medical event when based on appropriate medical judgement which may jeopardise the patient and may require medical or surgical intervention to prevent one of the other outcomes listed in the above definitions. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalisation or development of dependency or abuse.

5.2.6.1.3 Adverse events considered “Always Serious”

In accordance with the European Medicines Agency initiative on Important Medical Events, BI has set up a list of adverse events, which by their nature, can always be considered to be “serious” even though they may not have met the criteria of an SAE as defined above.

The latest list of “Always Serious Adverse Events” can be found in the Electronic Data Capture System and there is an algorithm in clinical database installed which detects non serious events which are per list definition serious. A copy of the latest list of “Always Serious Adverse Events” will be provided upon request. These events should always be reported as serious adverse events, as described in [Section 5.2.6.2](#).

Efficacy and safety endpoints are considered to be disease-related or are well-known side effects of fibrinolytics. These events are considered Outcome Events (OEs) and will need to be reported as serious adverse events on the SAE form if they meet criteria of an SAE and consequently will be reported in an expedited manner to the Competent Authorities.

Outcome Events are defined as follows:

Any bleeding including intracranial hemorrhage
All deaths
All myocardial infarctions
All strokes
All systemic embolisms
All transient ischemic attacks
All deep vein thromboses
All pulmonary embolisms

A standard narrative (if requested) and any required supporting documentation (including e.g. transesophageal echocardiogram [TEE], transthoracic echocardiogram [TTE], magnetic resonance tomography [MRT], computerized tomography, ECG examinations, or other) must also be provided for assessment of the DMC.

A key objective of the independent DMC is to protect patient safety by monitoring the incidence and clinical relevance of safety data including OEs, collected throughout the conduct of this study.

Cancers of new histology and exacerbations of existing cancer must be classified as a serious event regardless of the time since discontinuation of the drug and must be reported as described in [Section 5.2.6.2](#), subsections “AE Collection” and “**AE reporting to sponsor and timelines**”.

5.2.6.1.4 Adverse events of special interest

No adverse events of special interest have been defined and no DILI procedures are required for this trial as alteplase is a marketed product with well-known safety profile.

5.2.6.1.5 Intensity (severity) of Adverse events

The intensity (severity) of the AE should be judged based on the following:

Mild: Awareness of sign(s) or symptom(s) that is/are easily tolerated.
Moderate: Sufficient discomfort to cause interference with usual activity.
Severe: Incapacitating or causing inability to work or to perform usual activities.

.

5.2.6.1.6 Causal relationship of adverse events

Medical judgement should be used to determine whether there is a reasonable possibility of a causal relationship between the adverse event and the given study treatment, considering all relevant factors, including pattern of reaction, temporal relationship, de-challenge or re-challenge, confounding factors such as concomitant medication, concomitant diseases and relevant history.

Arguments that may suggest that there is a reasonable possibility of a causal relationship could be:

- The event is consistent with the known pharmacology of the drug.
- The event is known to be caused by or attributed to the drug class.
- A plausible time to onset of the event relative to the time of drug exposure.
- Evidence that the event is reproducible when the drug is re-introduced.
- No medically sound alternative aetiologies that could explain the event (e.g. pre-existing or concomitant diseases, or co-medications).
- The event is typically drug-related and infrequent in the general population not exposed to drugs (e.g. Stevens-Johnson syndrome).
- An indication of dose-response (i.e. greater effect size if the dose is increased, smaller effect size if dose is reduced).

Arguments that may suggest that there is no reasonable possibility of a causal relationship could be:

- No plausible time to onset of the event relative to the time of drug exposure is evident (e.g. pre-treatment cases, diagnosis of cancer or chronic disease within days/weeks of drug administration; an allergic reaction weeks after discontinuation of the drug concerned).

- Continuation of the event despite the withdrawal of the medication, taking into account the pharmacological properties of the compound (e.g. after 5 half-lives). Of note, this criterion may not be applicable to events whose time course is prolonged despite removing the original trigger.
- Additional arguments amongst those stated before, like alternative explanation (e.g. situations where other drugs or underlying diseases appear to provide a more likely explanation for the observed event than the drug concerned).
- Disappearance of the event even though the trial drug treatment continues or remains unchanged.

5.2.6.2 Adverse event collection and reporting

5.2.6.2.1 Adverse event collection

The investigator shall maintain and keep detailed records of all adverse events in the patient files. The following must be collected and documented on the appropriate eCRF(s) by the investigator:

- From signing the informed consent onwards until the individual patient's end of trial (the End of Study (EOS) Visit):
all adverse events (serious and non-serious).
- After the individual patient's end of trial:
the investigator does not need to actively monitor the patient for new adverse events but should only report any occurrence of cancer and trial treatment related serious adverse events of which the investigator may become aware of by any means of communication, e.g. phone call. Those adverse events should be reported on the BI SAE form (see Section 5.2.6.2.2), but not on the eCRF.

5.2.6.2.2 Adverse event reporting to the sponsor and timelines

The investigator must report serious adverse events, and non-serious adverse events which are relevant for the reported SAE on the BI SAE form via fax (except of Russia and UK where SAE form is to be send via Clinergize) immediately (within 24 hours) to the sponsor's unique entry point (country-specific contact details will be provided in the ISF). The same timeline applies if follow-up information becomes available. In specific occasions, the investigator could inform the sponsor upfront via telephone. This does not replace the requirement to complete and fax the BI SAE form.

With receipt of any further information to these events, a follow-up SAE form has to be provided. For follow-up information the same rules and timeline apply as for initial information. All serious and adverse events, including those persisting after individual patient's end of trial must be followed up until they have resolved, have been assessed as "chronic" or "stable", or no further information can be obtained.

5.2.6.2.3 Pregnancy

Not applicable for this trial. This trial will exclude pregnant women, and patients are severely ill during the observation period of the trial. To rule-out potential pregnancy urine pregnancy test is performed during screening and at Day 28.

5.3 DRUG CONCENTRATION MEASUREMENTS AND PHARMACOKINETICS

5.3.1 Assessment of pharmacokinetics

Participation in PK sampling is voluntary and not a prerequisite for participation in the trial. PK sampling will only occur after a separate PK informed consent has been given in accordance with local ethical and regulatory requirements. It is intended to obtain PK samples from at least 10 patients per dose group in Part 1, only. If PK samples collection will include less than 10 patients per treatment arm, collection of PK samples will also continue in Part 2.

Three samples per patient will be collected:

- 1st sample: prior to first drug administration on Day 1 (Visit 2a pre-dose) (planned time: -1:00 h; time window -2:00 h to just prior drug administration)
- 2nd sample: at steady state of the initial i.v. infusion on Day 1 (Visit 2a post-dose initial i.v. infusion) (planned time 1:30 h; time window: between 1 h after start of initial i.v. infusion and just prior to end of initial i.v. infusion)
- 3rd sample: at steady state of the long-term i.v. infusion on Day 1 (Visit 2a post-dose long-term i.v. infusion) (planned time: 6:00 h; time window: between 2 h after start of long-term i.v. infusion and just prior to end of long-term i.v. infusion)

The actual sampling date and time for blood samples will be reported in the eCRF. To allow a valid PK analysis, it is of utmost importance to document the exact clock time of start/end of infusion and blood sample collection. Every attempt should be made to adhere to a constant infusion rate of approximately 1 hour prior to drawing of sample 2 and approximately 2 hours prior to drawing of sample 3.

5.3.2 Methods of sample collection

For quantification of analyte plasma concentrations, blood will be taken from an antecubital or forearm vein, at the times indicated in the [Flow chart](#). Blood will be withdrawn by means of single-time venipuncture.

Details on sample characteristics, collection, processing, handling and shipment are provided in the Laboratory Manual in the ISF.

Samples will be stored in a freezer at the analytical laboratory until finalization of the clinical trial report (CTR). The plasma samples may be used for further methodological

investigations (e.g. for stability testing). However, only data related to the analyte will be generated by these additional investigations. The study samples will be discarded after completion of the additional investigations according to local legislation, but not later than at the time of completion of the CTR.

5.3.3 Analytical determinations

Plasma concentrations of alteplase will be determined by a validated immunoassay. All details of the analytical method will be available prior to the start of sample analysis.

5.4 ASSESSMENT OF BIOMARKERS

No exploratory biomarkers are planned. Established biomarkers of efficacy and safety are described and discussed in [Section 5.1](#) and [Section 5.2](#).

5.5 BIOBANKING

Not applicable.

5.6 OTHER ASSESSMENTS

Not applicable.

5.7 APPROPRIATENESS OF MEASUREMENTS

The primary endpoint measurements will be consistent with the recognized standard for ARDS in the setting of COVID-19 to assess treatment effects, including the the clinical functional status.

All secondary and further endpoint measurements performed during this trial are standard measurements and will be performed in order to determine the efficacy and safety in an appropriate way. The planned measurements are appropriate to see drug-induced changes in vital signs, functional parameters, standard laboratory values and coagulation parameters specific to efficacy and safety of the study drug. The endpoints are widely used and accepted for evaluation of efficacy and safety on an intravenously applied drug in a hospital setting.

Therefore, the appropriateness of all measurements applied in this trial is given.

6. INVESTIGATIONAL PLAN

The trial consists of three periods, a screening period, an intravenous open-label treatment period and a follow-up period.

During the treatment period, patients will receive an open-label infusion with alteplase on top of SOC, or SOC alone.

Instructions on alteplase infusion dosage and treatment schedule is provided in [Table 4.1.4:1](#).

All required procedures are described in the [Flow chart](#) and [Section 5](#).

After completion of the treatment period patients will be followed-up on Day 6, Day 7, Day 28 and Day 90/EOS.

If patient is discharged from hospital before completion of Day 28, the patient has to be invited to complete subsequent visits as per [Flow chart](#) until Day 90 including vital status collection.

6.1 VISIT SCHEDULE

No study procedures may be initiated prior to the patient or patient's legally acceptable representative signing the informed consent. A patient who could not provide informed consent at the start of the trial due to the severity of the disease, may have their legally acceptable representative to sign the consent on their behalf, in accordance with applicable laws and regulations. Once patient is recovered, they would need to provide their own consent to continue in the study.

An independent witness may be required in the event that the patient is able to give verbal consent, but unable to sign the consent form. Please refer to [Section 8.1](#) for more details on consenting process.

During the Screening Visit at any time of the day or night, eligible patients who are exhibiting signs and symptoms of COVID-19 induced ARDS may be asked to participate in the study.

Patients fulfilling all eligibility criteria will be randomised to one of three treatment arms in Part 1, and to one of two treatment arms in Part 2.

For potential modification of trial conduct in case of restrictions due to COVID-19, please refer to [Appendix 10.4](#).

6.2 DETAILS OF TRIAL PROCEDURES AT SELECTED VISITS

Study procedures to be performed at each visit are listed in the [Flow chart](#) and the respective protocol sections.

6.2.1 Screening and run-in periods

Screening Period

Once the patient has consented, the patient is considered to be enrolled in the trial. The patient should be recorded on the enrollment log and be registered in the IRT. Each patient will be assigned a unique patient number and enrollment will be recorded in eCRF.

For the schedule of assessments and procedures during the screening period refer to the [Flow Chart](#).

Laboratory samples shall be collected as per [Table 5.2.3:1](#) for haematology, clinical chemistry, renal function and coagulation including urine pregnancy testing (only applicable for women of childbearing potential). Local laboratories are intended to be used for all assessments.

The very first PaO₂/FiO₂ should be obtained preferably, when the patient is in a supine position.

SARS-CoV-2 positive test result has to be available before treatment administration.

Re-screening

A patient may be re-screened once within same hospital stay if the patient does not fulfill eligibility criteria. In case of re-screening patient will obtain new patient number per IRT.

Run-in Period

Not applicable.

6.2.2 Treatment periods

The patient is randomised (Day 1; Visit 2a) within 24 h after screening, information consent and confirmation of the eligibility criteria via IRT. Subsequently, study medication dispensing and administration will happen on the same day (Day 1), preferably within 6 hours of randomisation. Treatment period duration is 5 days.

Patients who discontinue trial treatment prematurely should still follow the trial schedule until Visit 6/EOS, if possible, please see [Section 3.3.4](#), including vital status collection.

For the schedule of assessments and procedures during the treatment period refer to the [Flow Chart](#).

Please refer to [Table 4.1.4:1](#) for Dosing and Treatment schedule and Instructions for Dose calculation and Infusion rates of Alteplase ([Appendix 10.5](#))

For details on PK sampling collection please refer to [Section 5.3.1](#).

During the first 6 days and during the stay on ICU (whatever is longer), PaO₂/FiO₂ should be measured at least three times daily in all patients, together with a recording of the patient's position.

Assessment of time-point of intubation and extubation, or start and end of non-invasive ventilation support is collected.

In addition to the scheduled ECGs, ECGs should be done and documented at any time of clinical event (e.g. acute event of arrhythmia, tachy- or bradycardia, angina, or MI).

6.2.3 Follow-up period and trial completion

Patients will be followed after treatment period up to Day 90. Follow-up period consists of 4 visits: Day 6, Day 7, Day 28 and Day 90/vital status collection.

For the schedule of assessments and procedures during the follow-up period refer to the [Flow Chart](#).

Vital status shall be collected for all patients at V6/EOS (see [Flow Chart](#)). Vital status can be collected by any means approved and accepted by local legislation in each individual country and does not require an on-site visit of the patient at the study site.

7. STATISTICAL METHODS AND DETERMINATION OF SAMPLE SIZE

The eligible patients for this trial will be randomised in Part 1 to one of three treatment arms

- alteplase 0.3mg/kg over 2 hours, followed by daily long-term (12h) infusion of 0.02 mg/kg/h over 5 days, "alteplase low"
- alteplase 0.6mg/kg over 2 hours, followed by daily long-term (12h) infusion of 0.04 mg/kg/h over 5 days, "alteplase high"
- standard of care

in a 1:1:1 ratio, stratified according to ventilation (invasive mechanical vs. non-invasive).

In Part 2, patients will be randomised to

- alteplase (one dosing regimen), depending on results of Part 1
- standard of care

in a 2:1 ratio, stratified according to ventilation (invasive mechanical vs. non-invasive)..

7.1 NULL AND ALTERNATIVE HYPOTHESES

Throughout this section, alteplase refers to the randomised treatment group, which is in addition to SOC. With regard to Part 1, reference will be made to alteplase low and alteplase high.

The primary endpoint related to Part 1, secondary endpoints, and further endpoints will be evaluated in an exploratory manner.

For Part 2:

The primary objective of the statistical analysis is to determine whether alteplase significantly increases the chances of clinical improvement or hospital discharge up to Day 28, in comparison to SOC alone. The Cox proportional hazards model will contain fixed effects for treatment, type of ventilation, baseline status of WHO scale and age, and will be used to estimate and test the hazard ratio of alteplase vs. SOC.

The null hypothesis tested by this trial is that the hazard ratio between alteplase and SOC equals 1 for all $t > 0$. The alternative hypothesis is that the hazard ratio between alteplase and SOC is not equal to 1 for all $t > 0$. Superiority will be declared if the hazard ratio between alteplase and SOC is significantly greater than 1 at the two-sided type I error level $\alpha = 5\%$.

7.2 PLANNED ANALYSES

7.2.1 General considerations

With regard to each efficacy and safety endpoint, the term "baseline" refers to the last observed measurement prior to randomisation for all patients.

The statistical analysis will be based on the following populations:

The Randomised Set (RS) will consist of all randomised patients.

The Full Analysis Set (FAS) will consist of all randomised patients with at least one baseline and one post baseline assessment relating to the primary endpoint of interest - refer to [Section 2.1.2](#) for primary endpoints related to Part 1 and Part 2.

The Treated Set (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.

Per Protocol Set (PPS) will consist of all patients in FAS who were without important protocol deviations (IPDs) for efficacy. The definition of IPDs will be specified in the Trial Statistical Analysis Plan (TSAP).

All endpoints will be presented for Parts 1 and Part 2 separately, and most endpoints will additionally be pooled for descriptive purposes.

7.2.2 Primary endpoint analyses

For primary endpoints related to Parts 1 and 2, refer to Section 2.1.2. The primary analyses will be performed on the FAS with treatment assignments as randomised.

Part 1

The primary endpoint for Part 1 will be analysed in a similar manner to Part 2, albeit in Part 1 this will be exploratory in nature, comparing alteplase low to SOC alone, and alteplase high to SOC alone.

Part 2

For the analysis of the primary endpoint of Part 2, patients that have not met the endpoint will be censored at Day 28 if they die prior to Day 28. Patients receiving bail-out therapy (as defined in [Section 4.2.2](#)) without having first met the endpoint, will be censored on the day of bail-out. This addresses a hypothetical estimand for the scenario of what would have happened had the bail-out not been administered. Since patients requiring bail-out would be expected to be those already deteriorating, this non-informative censoring could be considered a conservative approach to assessing this estimand. Patients who have not had clinical improvement by Day 28 will be censored on Day 28.

A cox proportional hazards model will be used to estimate the hazard ratio comparing the alteplase group to SOC. From this model, the 95% confidence interval and corresponding Wald p-value for this HR will be produced. The model will include fixed effects for treatment, type of ventilation, baseline status on the WHO eleven-point clinical progression scale and age. The Cox proportional hazard model assumptions will be checked.

Kaplan Meier estimates will be presented for this endpoint up to Day 28.

In anticipation of the rare situation where bail-out therapy may be used, as defined in [Section 4.2.2](#), two non-confirmatory supplementary analyses will be conducted, addressing the treatment policy estimand and a composite estimand whereby bail-out represents treatment failure and thereby, by definition, precludes any response after bail-out.

In the first of these supplementary analyses, addressing the treatment policy estimand, there will be no consideration for the intake of bail-out therapy.

In the second of these supplementary analyses, addressing the composite estimand, patients receiving bail-out therapy will be censored at Day 28, rather than on the day of bail-out.

7.2.3 Secondary endpoint analyses

For the list of secondary endpoints, refer to [Section 2.1.3](#). Summary statistics will be presented for each endpoint, and at each visit if appropriate.

Continuous endpoints will be analysed descriptively.

Binary endpoints will be analysed descriptively using frequency tables, risk differences and 95% confidence intervals.

More detailed descriptions will be provided in the TSAP.

7.2.4 Further endpoint analyses

The pharmacokinetic parameters listed in [Section 2.2](#) will be calculated according to the relevant standard operating procedure (SOP) of the Sponsor (001-MCS-36-472). Pharmacokinetic concentrations and further PK endpoints will be listed and analysed descriptively. Individual endogenous TPA will be determined for each patient at baseline (1st sample). Baseline levels will then be subtracted from all concentration measurements for that patient. In case the resulting concentration value is negative, it will be set to “BLQ”.

A population PK analysis and/or PK/PD analysis are planned to be conducted if sufficient data are available. In this case the results of such analysis may be reported in a separate, stand-alone pharmacometric report.

For the list of other further endpoints, refer to [Section 2.2.2](#). These further endpoints will be summarised descriptively, and in a similar manner to the primary and secondary endpoint analyses. Details on the analyses will be provided in the TSAP.

7.2.5 Safety analyses

Adverse events will be coded using the Medical Dictionary for Drug Regulatory Activities (MedDRA). Standard BI summary tables and listings will be produced. All adverse events with an onset between start of treatment and end of the REP, as defined in [Section 1.2](#), will be assigned to the on-treatment period for evaluation.

All treated patients will be included in the safety analysis. In general, safety analyses will be descriptive in nature and will be based on BI standards. No hypothesis testing is planned.

Statistical analysis and reporting of adverse events will concentrate on treatment-emergent adverse events, i.e. all adverse events occurring between start of treatment and end of the REP. Adverse events that start before first drug administration and deteriorate under treatment will also be considered as 'treatment-emergent'.

Frequency, severity, and causal relationship of adverse events will be tabulated by system organ class and preferred term after coding according to the current version of MedDRA at DBL.

Laboratory data will be analysed quantitatively and qualitatively. The latter will be done via comparison of laboratory data to their reference ranges. Values outside the reference range as well as values defined as clinically relevant will be summarised. Treatment arms will be compared descriptively with regard to distribution parameters as well as with regard to frequency and percentage of patients with abnormal values or clinically relevant abnormal values.

Vital signs, physical examinations, or other safety-relevant data observed at screening, baseline, during the course of the trial and at the end-of-trial evaluation will be assessed with regard to possible changes compared with findings before start of treatment.

Further details on the safety analysis will be specified in the TSAP.

7.2.6 Interim Analyses

One interim analysis is planned after the first (approximately) 60 patients (Part 1). An independent DMC will review the efficacy and safety data and provide a recommendation regarding the continuation of the trial into Part 2 and the corresponding dose for the alteplase treatment arm. The DMC charter will lay out the general principles of decision-making of the DMC. Further details on the DMC are described in [Section 8.7](#). Data from Part 1 of the SOC group and of the continued alteplase arm will not be included in the final primary analysis.

7.3 HANDLING OF MISSING DATA

For the analysis of the primary endpoint, time to clinical improvement or hospital discharge up to Day 28, patients will be censored at Day 28 if they die prior to Day 28, at the time they received bail-out therapy, or have not had clinical improvement by Day 28.

With respect to safety evaluations, it is not planned to impute missing values.

7.4 RANDOMISATION

BI will arrange for the randomisation and the packaging and labelling of trial medication. The randomisation list will be generated using a validated system, which involves a

pseudo-random number generator so that the resulting treatment will be reproducible and non-predictable. The block size will be documented in the CTR. Access to the codes will be controlled and documented.

Randomisation in Part 1 will be to the three treatment arms of:-

- alteplase 0.3mg/kg over 2 hours followed by 0.02 mg/kg/h over 12 hours “alteplase low”
- alteplase 0.6 mg/kg over 2 hours followed by 0.04 mg/kg/h over 12 hours “alteplase high”
- SOC

With an allocation ratio of 1:1:1, with stratification by the following factor:

- Type of ventilation (non-invasive or invasive mechanical)

Randomisation in Part 2 will be to the one alteplase arm and to standard of care, with an allocation ratio of 2:1, with stratification by the following factor:

- Type of ventilation (non-invasive or invasive mechanical)

7.5 DETERMINATION OF SAMPLE SIZE

Part 1 of the study plans to randomise approximately 60 patients in total, with a view to exploring the data in a descriptive manner.

Part 2 has a planned confirmatory analysis at the 2-sided alpha level of 0.05 with a power of approximately 80% and with a planned review of the assumptions at the end of Part 1. With a randomisation allocation ratio of 2:1, this leads to approximately 140 patients on the treatment arm and approximately 70 patients on the SOC alone. This is assuming a hazard ratio of 1.6 with median time to clinical improvement rates of 10 and 16 days, and event rates of 85% and 70% for the treatment arm and SOC respectively. This is also in line with the null hypothesis as defined in [Section 7.1](#), whereby it is planned to test for superiority of alteplase vs SOC alone. Whilst the assumptions of the hazard ratio, event rates and median times to response are still rather approximate, the magnitude of these assumptions is similar to the available results in other COVID-19 patients [[R20-2001](#), [R20-2246](#)].

The table below presents other scenarios for consideration, whereby the resulting power ranges from approximately 63% to 88%.

Table 7.5:1 Power calculations with 2-sided $\alpha = 0.05$, 2:1 allocation and 210 patients

Hazard ratio (alteplase vs SOC alone)	1.45	1.60	1.67
Event rate for SOC alone	73%	70%	68%
Event rate for alteplase	85%	85%	85%
Median time to event for SOC alone	15 days	16 days	17 days
Median time to event for alteplase	10 days	10 days	10 days
Total number of events	170	168	167
Power	63%	82%	88%

Calculations were performed using the software R Version 3.6.1.

Only patients randomised into Part 1 will be included in the analyses of Part 1, and only patients randomised into Part 2 will be included in the confirmatory analyses of Part 2.

8. INFORMED CONSENT, TRIAL RECORDS, DATA PROTECTION, PUBLICATION POLICY, AND ADMINISTRATIVE STRUCTURE

The trial will be carried out in compliance with the protocol, the ethical principles laid down in the Declaration of Helsinki, in accordance with the ICH Harmonized Guideline for GCP, relevant BI SOPs, the EU directive 2001/20/EC / EU regulation 536/2014 and other relevant regulations. Investigators and site staff must adhere to these principles. Deviation from the protocol, the principles of ICH-GCP or applicable regulations as will be treated as “protocol deviation”.

Standard medical care (prophylactic, diagnostic and therapeutic procedures) remains the responsibility of the treating physician of the patient.

The investigator should inform the sponsor immediately of any urgent safety measures taken to protect the trial patients against any immediate hazard, as well as of any serious breaches of the protocol or of ICH-GCP.

The BI transparency and publication policy can be found on the following web page: <https://trials.boehringer-ingelheim.com/>. The rights of the investigator and of the sponsor with regard to publication of the results of this trial are described in the investigator contract. As a rule, no trial results should be published prior to finalisation of the CTR.

The certificate of insurance cover is made available to the investigator and the patients, and is stored in the ISF.

8.1 TRIAL APPROVAL, PATIENT INFORMATION, INFORMED CONSENT

This trial will be initiated only after all required legal documentation has been reviewed and approved by the respective IRB / EC and Competent Authority (CA) according to national and international regulations. The same applies for the implementation of changes introduced by amendments.

Prior to patient participation in the study, each prospective subject or their legally acceptable representative (in accordance with national legislation and ICH GCP) will be provided with all information relating to the study and a copy of the patient information and consent form.

Once the essential study information has been provided and the investigator is assured that each subject, or their legally acceptable representative, understands the implications of participating in the study, the subject, or their legally acceptable representative, will be asked to give consent to participate in the study by signing the informed consent form.

An independent impartial witness may be required in the event that the patient is able to give verbal consent, but unable to sign the consent form. In this case, the independent impartial witness will sign a form to indicate that the information was provided to the patient, the

patient appeared to understand the information and gave consent to participate in the study. The consent from the subject may be given verbally or by means of an unequivocal sign such as nodding or raising their hand in response to a question. The method used must be approved by the ethics committee and documented in the medical records.

If a short written informed consent document is initially used, once the patient recovers the investigator or delegate must discuss the study and sign the full patient consent and information sheet with the patient in order for patient to continue in the study. A note of the type of written informed consent obtained will be made in the subject's medical history/patient notes and recorded in the CRF.

An optional assessment of pharmacokinetics will also be performed in at least 10 patients per dose group in Part 1, only. This pharmacokinetic sampling is voluntary and is not a prerequisite for study participation. Sampling will only occur after a separate pharmacokinetic informed consent has been given in accordance with the local ethics and regulatory requirements. Please refer to [Section 5.3.1](#) for more details on pharmacokinetic sampling process.

All signatures must be dated by each signatory and all completed consent forms will need to be retained by the Investigator and copies provided to the subject or the subject's legally acceptable representative.

Re-consenting may become necessary when new relevant information becomes available and should be conducted according to the sponsor's instructions. The consent and re-consenting process should be properly documented in the source documentation.

8.2 DATA QUALITY ASSURANCE

A risk-based approach is used for trial quality management. It is initiated by the assessment of critical data and processes for trial patient protection and reliability of the results as well as identification and assessment of associated risks. An Integrated Quality and Risk Management Plan documents the rationale and strategies for risk management during trial conduct including monitoring approaches, vendor management and other processes focusing on areas of greatest risk.

Continuous risk review and assessment may lead to adjustments in trial conduct, trial design or monitoring approaches.

A quality assurance audit/inspection of this trial may be conducted by the sponsor, sponsor's designees, or by IRB / EC or by regulatory authorities. The quality assurance auditor will have access to all medical records, the investigator's trial-related files and correspondence, and the informed consent documentation of this clinical trial.

8.3 RECORDS

Electronic CRFs for individual patients will be provided by the sponsor. See [Section 4.1.5.2](#) for rules about emergency code breaks. For drug accountability, refer to [Section 4.1.8](#).

8.3.1 Source documents

In accordance with regulatory requirements, the investigator should prepare and maintain adequate and accurate source documents and trial records that include all observations and other data pertinent to the investigation on each trial patient. Source data as well as reported data should be **attributable, legible, contemporaneous, original and accurate (ALCOA)**. Changes to the data should be traceable (audit trail).

Data reported on the eCRF must be consistent with the source data or the discrepancies must be explained.

The current medical history of the patient may not be sufficient to confirm eligibility for the trial and the investigator may need to request previous medical histories and evidence of any diagnostic tests. In this case, the investigator must make at least one documented attempt to retrieve previous medical records. If this fails, a verbal history from the patient or his legal representative, documented in their medical records, would be acceptable.

The investigator must ensure that all patient identifiers (e.g. patient's name, initials, address, phone number, social security number) have properly been removed or redacted from any copy of the patients' source documents before sending them to the sponsor.

If the patient is not compliant with the protocol, any corrective action (e.g. re-training) must be documented in the patient file.

Due to Covid-19 restrictions study conduct, including site monitoring and access to source documents, may need to be adjusted accordingly. See [Appendix 10.4](#) for further details.

For the eCRF, data must be derived from source documents, for example:

- Patient identification: gender, year of birth (in accordance with local laws and regulations)
- Patient participation in the trial (substance, trial number, patient number, date patient was informed)
- Dates of patient's visits, including dispensing of trial medication
- Medical history (including trial indication and concomitant diseases, if applicable)
- Medication history
- Adverse events and OEs onset date (mandatory), and end date (if available)
- Serious adverse events onset date (mandatory), and end date (if available)
- Concomitant therapy (start date, changes)
- Originals or copies of laboratory results and other imaging or testing results, with proper documented medical evaluation (in validated electronic format, if available)
- Completion of patient's participation in the trial" (end date; in case of premature discontinuation document the reason for it).
- Prior to allocation of a patient to a treatment into a clinical trial, there must be documented evidence in the source data (e.g. medical records) that the trial participant meets all inclusion criteria and does not meet any exclusion criteria. The absence of

records (either medical records, verbal documented feedback of the patient or testing conducted specific for a protocol) to support inclusion/exclusion criteria does not make the patient eligible for the clinical trial.

8.3.2 Direct access to source data and documents

During the conduct of the study, it may not be possible for the investigator / institution to allow site trial-related monitoring, audits, IRC/EC review or regular inspections due to COVID-19. However, once site visits are allowed again, the site will need to provide a direct access to the eCRF and all source documents/data, including progress notes, copies of laboratory and medical test results, which must be available at all times for review by the CRA, auditor and regulatory inspector (e.g. FDA). They may review all eCRFs and informed consents. The accuracy of the data will be verified by direct comparison with the source documents described in [Section 8.3.1](#). The sponsor will also monitor compliance with the protocol and GCP.

8.3.3 Storage period of records

Trial sites:

The trial sites must retain the source and essential documents (including ISF) according to contract or the local requirements valid at the time of the end of the trial (whatever is longer).

Sponsor:

The sponsor must retain the essential documents according to the sponsor's SOPs.

8.4 EXPEDITED REPORTING OF ADVERSE EVENTS

BI is responsible to fulfil their legal and regulatory reporting obligation in accordance with regulatory requirements.

8.5 STATEMENT OF CONFIDENTIALITY AND PATIENT PRIVACY

Data protection and data security measures are implemented for the collection, storage and processing of patient data in accordance with the Principles 7 and 12 of the WHO GCP handbook.

Individual patient data obtained as a result of this trial is considered confidential and disclosure to third parties is prohibited with the following exceptions:

- Personalised treatment data may be given to the patient's personal physician or to other appropriate medical personnel responsible for the patient's welfare.
- Data generated at the site as a result of the trial need to be available for inspection on request by the participating physicians, the sponsor's representatives, by the IRB / EC and the regulatory authorities.

8.5.1 Collection, storage and future use of biological samples and corresponding data

Measures are in place to comply with the applicable rules for the collection, storage and future use of biological samples and clinical data, in particular:

- The BI-internal facilities storing biological samples from clinical trial participants as well as the laboratories' activities for clinical trials sponsored by Boehringer Ingelheim are regularly audited and qualified for the storage of biological samples collected in clinical trials.
- An appropriate sample and data management system, including audit trail for clinical data and samples to identify and destroy such samples according to ICF is in place.
- A fit for the purpose documentation (analysis plan and report) ensures compliant usage.
- Samples and data are used only if an appropriate informed consent is available.

8.6 TRIAL MILESTONES

The start of the trial is defined as the date when the FPI, in the whole trial, signs informed consent.

The end of the trial is defined as the date of the last visit of the last patient in the whole trial ("Last Patient Completed").

The "**Last Patient Last Treatment**" (LPLT) date is defined as the date on which the last patient in the whole trial is administered the last dose of trial treatment (as scheduled per protocol or prematurely). Individual investigators will be notified of SUSARs occurring with the trial medication until 30 days after LPLT at their site.

Early termination of the trial is defined as the premature termination of the trial due to any reason before the end of the trial as specified in this protocol.

Temporary halt of the trial is defined as any unplanned interruption of the trial by the sponsor with the intention to resume it.

Suspension of the trial is defined as an interruption of the trial based on a Health Authority request.

The EC / Competent Authority in each participating EU member state will be notified about the trial milestones according to the respective laws.

A final report of the clinical trial data will be written only after all patients have completed the trial in all countries (EU or non-EU) to incorporate and consider all data in the report. The sponsor will submit to the EU database a summary of the final trial results within one year from the end of a clinical trial as a whole, regardless of the country of the last patient (EU or non-EU).

8.7 ADMINISTRATIVE STRUCTURE OF THE TRIAL

The trial is sponsored by BI.

A Coordinating Investigator is responsible to coordinate investigators at the different sites participating in this trial. Tasks and responsibilities are defined in a contract.

A Steering Committee (SC) consisting of independent experts and sponsor representatives will be established to support the Coordinating Investigator who will be the chair of the SC. The composition of the SC will be documented in the Trial Master File. The tasks and responsibilities will be agreed in contracts between the SC members and the sponsor and also summarised in a SC Charter.

A DMC will be established. Members of the DMC are independent of BI, they are physicians experienced in the treatment of the disease under investigation and/or thrombolytic treatments and a statistician.

The DMC will evaluate safety and efficacy data as well as the results of the interim analysis. The DMC will receive urgent significant safety events, including cases of intracranial haemorrhage and fatal bleeds for immediate evaluation. Regular DMC meetings will be held at specified intervals. The DMC will recommend continuation, modification or termination of the trial as detailed in the DMC charter. DMC recommendations as well as the final BI decision will be reported to the appropriate Regulatory Authorities (RAs)/Health Authorities (HAs), IRBs/ECs, and to investigators as requested by local law. The tasks and responsibilities of the DMC are specified in a charter.

Relevant documentation on the participating (Principal) Investigators (e.g. their curricula vitae) will be filed in the ISF.

The investigators will have access to the BI web portal Clinergize to access documents provided by the sponsor.

BI has appointed a Clinical Trial Leader (CT Leader), responsible for coordinating all required activities, in order to

- manage the trial in accordance with applicable regulations and internal SOPs,
- direct the clinical trial team in the preparation, conduct, and reporting of the trial,
- ensure appropriate training and information of Clinical Trial Managers, CRAs, and investigators of participating countries.

The organisation of the trial in the participating countries will be performed by the respective local or regional BI-organisation (Operating Unit, OPU) in accordance with applicable regulations and BI SOPs, or by a CRO with which the responsibilities and tasks will have been agreed and a written contract filed before initiation of the clinical trial.

Data Management and Statistical Evaluation will be done by BI according to BI SOPs.

Tasks and functions assigned in order to organise, manage, and evaluate the trial are defined according to BI SOPs. A list of responsible persons and relevant local information can be found in the ISF.

An IRT vendor will be used in this trial. Details will be provided in the IRT Manual and, available in the ISF.

9. REFERENCES

9.1 PUBLISHED REFERENCES

- P00-10646 Gram J, Münster AM, Dilling-Hansen B, Al Lavassani H, Lahoz AX, Jespersen J. Inhalation/intravenous recombinant tissue plasminogen activator and inhaled heparin in a patient with acute respiratory distress syndrome. *Fibrinolysis and Proteolysis*. 1999. 13(4/5): 209-212.
- P08-12177 Hacke W, Kaste M, Bluhmki E, Brozman M, Davalos A, Guidetti D, Larrue V, Lees KR, Medeghri Z, Machnig T, Schneider D, Kummer R von, Wahlgren N, Toni D, ECASS Investigators. Thrombolysis with alteplase 3 to 4.5 hours after acute ischemic stroke. *N Engl J Med* 2008; 359(13): 1317-1349.
- P08-16186 Hofstra JJ, Haitzma JJ, Juffermans NP, Levi M, Schultz MJ. The role of bronchoalveolar hemostasis in the pathogenesis of acute lung injury. *Seminars in thrombosis and hemostasis*. 2008. 34(5):475-484.
- P12-04032 Sebag SC, Bastarache JA, Ware LB. Therapeutic modulation of coagulation and fibrinolysis in acute lung injury and the acute respiratory distress syndrome. *Curr Pharm Biotechnol*. 2011. 12(9): 1481–1496.
- P13-04526 Hofstra JJ, Cornet AD, Declerck PJ, Dixon B, Aslami H, Vlaar APJ, et al. Nebulized fibrinolytic agents improve pulmonary fibrinolysis but not inflammation in rat models of direct and indirect acute lung injury. *PLoS One*. 2013. 8(2): e55262.
- P13-13102 Skeik N, Gits CC, Ehrenwald E, Cragg AH Fibrinogen level as a surrogate for the outcome of thrombolytic therapy using tissue plasminogen activator for acute lower extremity intravascular thrombosis. *Vasc Endovascular Surg*. 2013 47(7): 519-23. .
- P15-13160 Lee K, Istl A, Dubois L, DeRose G, Forbes TL, Wiseman D, et al. Fibrinogen level and bleeding risk during catheter-directed thrombolysis using tissue plasminogen activator. *Vasc Endovascular Surg*. 2015. 49(7): 175-179.
- P17-13366 Vedantham S, Goldhaber SZ, Julian JA, Kahn SR, Jaff MR, Cohen DJ, et al. Pharmacomechanical catheter-directed thrombolysis for deep-vein thrombosis. *N Engl J Med*. 2017. 377(23): 2240-2252.
- P20-03126 Moore HB, Barrett CD, Moore EE, McIntyre RC, Moore PK, Talmor DS, et al. Is there a role for tissue plasminogen activator (rt-Part-PA) as a novel

- treatment for refractory COVID-19 associated acute respiratory distress syndrome (ARDS)? J Trauma Acute Care Surg. 2020. 88(6): 713-714.
- P20-04176 Bikdeli B, Madhavan MV, Jimenez D, Chuich T , Dreyfus I , Driggin E, et al. COVID-19 and thrombotic or thromboembolic disease: implications for prevention, antithrombotic therapy, and follow-up: JACC state-of-the-art review. J Am Coll Cardiol. 2020. 75(23): 2950-2973.
- P20-04591 Barrett CD, Oren-Grinberg A, Chao E, Moraco AH, Martin MJ, Reddy SH, et al. Rescue therapy for severe COVID-19 associated acute respiratory distress syndrome (ARDS) with tissue plasminogen activator (rt-Part-PA): a case series. J Trauma Acute Care Surg. 2020.
- P20-04704 Poor HD, Ventetuolo CE, Tolbert T, Chun G, Serrao G, Zeidman A, et al. COVID-19 Critical illness pathophysiology driven by diffuse pulmonary thrombi and pulmonary endothelial dysfunction responsive to thrombolysis. Clin. Transl. Med. Accepted Author Manuscript. doi:10.1002/ctm2.44
- P85-16126 Nilsson T, Wallen P, Mellbring G. In vivo metabolism of human tissue-type plasminogen activator. Scand J Haematol. 1984. 33: 49-53.
- P86-5233 Verstraete M, Su CAPF, Tanswell P, Feuerer W, Collen D. Pharmacokinetics and effects on fibrinolytic and coagulation parameters of two doses of recombinant tissue-type plasminogen activator in healthy volunteers. Thromb Haemost. 1986. 56(1): 1-5.
- P89-46753 Seifried E, Tanswell P, Ellbrueck D, Haerer W, Schmidt A. Pharmacokinetics and haemostatic status during consecutive infusions of recombinant tissue-type plasminogen activator in patients with acute myocardial infarction. Thromb Haemost. 1989. 61(3): 497-501.
- P89-49823 Tanswell P, Seifried E, Su CAPF, Feuerer W, Rijken DC. Pharmacokinetics and systemic effects of tissue-type plasminogen activator in normal subjects. Clin Pharmacol Ther. 1989. 46(2): 155-162.
- P90-53645 Urano T, Sakakibara K, Takada Y, Takada A. Stimulation of the amidolytic activity of single chain tissue plasminogen activator by fibrinogen and its degradation products. 12th Cong of the Int Society on Thrombosis and Haemostasis, Tokyo, Aug 19 - 25 1989. Thromb Haemost. 1989. 62(1): 84.
- P90-52475 Simank HG, et al. Clinical usefulness of D-dimer: Evaluation during fibrinolytic treatment of venous thrombosis or myocardial infarction. Thromb Res 1989 ; 56(4) ; 541-546.

- P91-62566 Verstraete M, et al. Effects of alteplase and saruplase on hemostatic variables: A single-blind, randomized trial in patients with acute myocardial infarction. *Coronary Artery Dis* 1991 ; 2(3) ; 349-355.
- P92-71704 Klein P, et al. Changes of systemic fibrinolytic and hemostatic parameters during local fibrinolysis with tissue-plasminogen activator (rt-PA). *Vasa* 1992 ; 21(3) ; 249-252.
- P93-77527 Topol E. An international, randomized trial comparing four thrombolytic strategies for acute myocardial infarction. *N Engl J Med* 1993.329(10); 673-682.
- P94-80931 Idell S, James KK, Coalson JJ. Fibrinolytic activity in bronchoalveolar lavage of baboons with diffuse alveolar damage: trends in two forms of lung injury. *Crit Care Med*. 1992. (10): 1431-1440.
- P96-2358 Nordt TK, Moser M, Peter K, Kohler B, Bode C. Early thrombocyte-hyperaggregation during thrombolysis for acute myocardial infarction as a risk factor for late reocclusion. 62nd Ann Mtg of the German Society for Cardiology - Heart and Circulation Research, Mannheim, 11 – 13 Apr 1996. *Z Kardiol*. 1996. 85(Suppl 2): 284.
- P98-10462 Gurwitz JH, Gore JM, Goldberg RJ, Barron HV, Breen T, Rundle AC, et al. Risk for intracranial hemorrhage after tissue plasminogen activator treatment for acute myocardial infarction. Participants in the National Registry of Myocardial Infarction 2. *Annals of internal medicine*. 1998. 129(8): 597-604.
- P99-02520 Werf F van de. Single-bolus tenecteplase compared with front-loaded alteplase in acute myocardial infarction: the ASSENT-2 double-blind randomised trial. *Lancet*. 1999. 354 (9180): 716-722.
- R05-0344 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.
- R20-1309 Guan WJ, Ni ZY, Hu Y, Liang WH, Ou CQ, He JX, et al. Clinical characteristics of coronavirus disease 2019 in China. *N Engl J Med*. 2020.
- R20-1310 Yang X, Yu Y, Xu J, Shu H, Xia J, Liu H, et al. Clinical course and outcomes of hospitalized patients with SARS-CoV-2 pneumonia in Wuhan, China: a single-centered, retrospective, observational study. *Lancet Respir Med*. 2020.

- R20-1366 Xu Z, Shi L, Wang Y, Zhang J, Huang L, Zhang C, et al. Pathological findings of COVID-19 associated with acute respiratory distress syndrome. *Lancet Respir Med*. 2020. 8(4): 420-422.
- R20-1643 Tian S, Hu W, Niu L, Liu H, Xu H, Xiao SY. Pulmonary pathology of early-phase 2019 novel coronavirus (COVID-19) pneumonia in two patients with lung cancer. *J Thorac Oncol*. 2020.
- R20-1647 Acute Respiratory Distress Syndrome Network, Brower RG, Matthay MA, et al. Ventilation with lower tidal volumes as compared with traditional tidal volumes for acute lung injury and the acute respiratory distress syndrome. *N Engl J Med*. 2000. 342(18): 1301-1308.
- R20-1649 Ackermann M, Verleden SE, Kuehnel M, Haverich A, Welte T, Laenger F, et al. Pulmonary vascular endothelialitis, thrombosis, and angiogenesis in COVID-19. *N Engl J Med*. 2020.
- R20-1650 Hardaway RM, Harke H, Tyroch AH, Williams CH, Vazquez Y, et al. Treatment of severe acute respiratory distress syndrome: a final report on a phase I study. *The American surgeon*. 2001. 67(4): 377-382.
- R20-1652 Aranda-Valderrama P, Kaynar AM. The basic science and molecular mechanisms of lung injury and acute respiratory distress syndrome. *Int Anesthesiol Clin*. 2018. 56(1):1-25.
- R20-1653 Barrett CD, Moore HB, Banerjee A, Silliman CC, Moore EE, Yaffe MB. Human neutrophil elastase mediates fibrinolysis shutdown through competitive degradation of plasminogen and generation of angiostatin. *The journal of trauma and acute care surgery*. 2017. 83(6): 1053-61.
- R20-1654 Bhargava M, Wendt CH. Biomarkers in acute lung injury. *Translational research : the journal of laboratory and clinical medicine*. 2012. 159(4): 205-17.
- R20-1655 Tang N, Li D, Wang X, Sun Z. Abnormal coagulation parameters are associated with poor prognosis in patients with novel coronavirus pneumonia. *Journal of thrombosis and haemostasis : JTH*. 2020.
- R20-1656 Liu C, Ma Y, Su Z, Zhao R, Zhao X, Nie HG, et al. Analysis of preclinical studies of fibrinolytic therapy for acute lung injury. *Front Immunol*. 2018. 20(9):1898.
- R20-1695 Lopes Ferreira F, Peres Bota D, Bross A, Melot C, Vincent JL. Serial evaluation of the SOFA score to predict outcome in hospitalized patients. *JAMA*. 2001. 286(14):1754-1758.

- R20-1696 Knaus WA, Draper EA, Wagner DP, Zimmerman JE APACHE II: a severity of disease classification system. *Crit Care Med.* 1985. 13(10): 818-29.
- R20-1697 Teasdale G, Maas A, Lecky F, Manley G, Stocchetti N, Murray G The Glasgow Coma Scale at 40 years: standing the test of time. *Lancet Neurol.* 2014. 13(8): 844-54.
- R20-1709 Brown SM, Duggal A, Hou PC, Tidswell M, Khan A, Exline M, et al, Non-linear imputation of PaO₂/FiO₂ from SpO₂/ FiO₂ among mechanically ventilated patients in the intensive care unit: a prospective, observational study. *Crit Care Med.* Author manuscript; available in PMC 2018 Aug 1.
- R20-1710 Rapsang AG, Shyam DC. Scoring systems in the intensive care unit: A compendium. *Indian J Crit Care Med.* 2014. 18(4): 220–228.
- R20-1760 Guenther A, Mosavi P, Heinemann S, Ruppert C, Muth H, Markart P, et al. Alveolar fibrin formation caused by enhanced procoagulant and depressed fibrinolytic capacities in severe pneumonia. *Am J Respir Crit Care Med.* 2000. 161: 454–462.
- R20-1804 Llitjos JF, Leclerc M, Chochois C, Monsallier MJ, Ramakers M, Auvray M, et al. High incidence of venous thromboembolic events in anticoagulated severe COVID-19 patients. *J Thromb Haemost.* 2020. 22;10.
- R20-1851 Szekely Y, Lichter Y, Taieb P, Banai A, Hochstadt A, Merdler I, et al. The spectrum of cardiac manifestations in coronavirus disease 2019 (COVID-19) – a systematic echocardiographic study. *Circulation* Originally published 29 May 2020, published ahead of print, doi: 10.1161/CIRCULATIONAHA.120.047971; 2020
- R20-1852 Paranjpe I, Fuster V, Lala A, Russak A, Glicksberg BS, Levin MA, et al. Association of treatment dose anticoagulation with in-hospital survival among hospitalized patients with COVID-19. *Journal of the American College of Cardiology, Journal Pre-proof*, online 6 May 2020, doi:10.1016/j.jacc.2020.05.001; 2020
- R20-1934 Mahmoud AAA, Mahmoud HE, Mahran MA, Khaled M. Steptokinase versus unfractionated heparin nebulization in patients with severe acute respiratory distress syndrome (ARDS): A randomized controlled trial with observational controls. *Journal of Cardiothoracic and Vascular Anesthesia.* 2020. 34(436): 443.

- R20-1941 Puelles VG, Luetgehetmann M, Lindenmeyer MT, Sperhake JP, Wong MN, Allweiss L, et al, Multiorgan and renal tropism of SARS-CoV-2. New England Journal of Medicine, published on May 13, 2020, doi: 10.1056/NEJM c2011400; 2020.
- R20-1952 Ciceri F, Beretta L, Scandroglio AM, Colombo S, Landoni G, Ruggeri A, et al. Microvascular COVID-19 lung vessels obstructive thromboinflammatory syndrome (MicroCLOTS): an atypical acute respiratory distress syndrome working hypothesis. Crit Care Resusc. 2020. 22(2): 95-97.
- R20-1954 Oxford University News Release. Low-cost dexamethasone reduces death by up to one third in hospitalised patients with severe respiratory complications of COVID-19. 16 June 2020.
- R20-2001 Cao B, Wang Y, Wen D, Liu W, Wang J, Fan G, et al. A trial of lopinavir-ritonavir in adults hospitalized with severe Covid-19. N Engl J Med 2020;382(19):1787-1799.
- R20-2002 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.
- R20-2246 Grein J, Ohmagari N, Shin D, Diaz G, Asperges E, Castagna A, et al. Compassionate use of remdesivir for patients with severe Covid-19. N Engl J Med 2020;382(24):2327-2336.
- R96-2065 Magnusson S, Petersen TE, Sottrup-Jensen L, Claeys H. Complete primary structure of prothrombin: isolation, structure and reactivity of ten carboxylated glutamic acid residues and regulation of prothrombin activations by thrombin. In: Reich E, Rifkin DB, Shaw E, editors. Proteases and Biological Control. New York: Cold Spring Harbor Laboratory; 1975. p. 123-149

9.2 UNPUBLISHED REFERENCES

- c32479735-01 Simulations in support of dose and schedule selection for alteplase in patients with ARDS applying a mathematical pharmacokinetic/pharmacodynamic model for fibrinolytic proteins.
- c32361824 Investigator brochure (current version)
- U85-0697 Verstraete M, Collen D. Report on a pharmacokinetic study of recombination human tissue type plasminogen activator (RT-PA) (G11021). 1985

- U86-0354 Tanswell P. Pharmacokinetics of recombinant tissue type plasminogen activator (0.25 mg/kg) after 10 minute intravenous infusion of 2 different formulations in healthy volunteers. 1986
- U87-0368 Su CAPF. Pharmacokinetics, pharmacodynamics and tolerance of a 30 minute infusion of 0.25 mg/kg bodyweight and 0.50 mg/kg bodyweight of rt-PA in healthy volunteers. 1987
- U87-0935 Baughman R, Benninger A, Grossbard E. A pharmacokinetic and safety study of recombinant human tissue type plasminogen activator in human volunteers. 1985
- U95-2167 Haarmann W., Bluhmki E., Efficacy of fibrinolytic therapy with rt-PA in patients with deep vein thrombosis of lower and upper extremities. 1995
- U99-0193 Danays T. A phase III, randomized, double-blind, parallel-group, international trial of single bolus of TNK-tissue plasminogen activator (TNK-tPA) VS accelerated infusion of rt-PA (Alteplase: Activase, Actilyse) in acute myocardial infarction: Assent II (Assessment of the safety and efficacy of a new thrombolytic agent). 19 June 1999
- U99-1584 W. Haarmann. E. Bluhmki, G. Slawik Comparison of the efficacy and safety of intravenous locoregional administration of 20 mg rt-PA, 40 mg rt-PA, compared intravenous, systemic administration of 9 million units Streptokinase given over 6 hours given up to 5 days in patients with deep vein thrombosis of the lower extremities. 30 June 1999.

10. APPENDICES

10.1 GLASGOW COMA SCALE

The GCS score [[R20-1697](#), [R20-1710](#)] is calculated as follows:

Score	Best eye response (E)
1	No eye opening
2	Eye opening to pain
3	Eye opening to verbal command
4	Eyes open spontaneously
Score	Best verbal response (V)
1	No verbal response
2	Incomprehensible sounds
3	Inappropriate words
4	Confused
5	Oriented
Score	Best motor response (M)
1	No motor response
2	Extension to pain
3	Flexion to pain
4	Withdrawal from pain
5	Localizing pain
6	Obeys commands

A coma score of 13 or higher correlates with a mild brain injury, 9-12 is a moderate injury and 8 or less a severe brain injury

10.2 SOFA AND APACHE II SCORES

The SOFA score [[R20-1695](#); [R20-1710](#)] is calculated as follows:

Variables	SOFA Score				
	0	1	2	3	4
Respiratory Pao ₂ /Fio ₂ , mm Hg	>400	≤400	≤300	≤200†	≤100†
Coagulation Platelets ×10 ⁹ /μL‡	>150	≤150	≤100	≤50	≤20
Liver Bilirubin, mg/dL‡	<1.2	1.2-1.9	2.0-5.9	6.0-11.9	>12.0
Cardiovascular Hypotension	No hypotension	Mean arterial pressure <70 mm Hg	Dop ≤5 or dob (any dose)§	Dop >5, epi ≤0.1, or norepi ≤0.1§	Dop >15, epi >0.1, or norepi >0.1§
Central nervous system Glasgow Coma Score Scale	15	13-14	10-12	6-9	<6
Renal Creatinine, mg/dL or urine output, mL/d	<1.2	1.2-1.9	2.0-3.4	3.5-4.9 or <500	>5.0 or <200

*Norepi indicates norepinephrine; Dob, dobutamine; Dop, dopamine; Epi, epinephrine; and Fio₂, fraction of inspired oxygen.

†Values are with respiratory support.

‡To convert bilirubin from mg/dL to μmol/L, multiply by 17.1.

§Adrenergic agents administered for at least 1 hour (doses given are in μg/kg per minute).

||To convert creatinine from mg/dL to μmol/L, multiply by 88.4.

APACHE II score

The APACHE II [[R20-1696](#); [R20-1710](#)] score is calculated as follows:

A: Acute physiological score (12 variables)									
Physiologic variable	High abnormal range				Normal range	Low abnormal range			
	+4	+3	+2	+1		+1	+2	+3	+4
Temperature rectal (°C)	≥41	39-40.9	-	38.5-38.9	36-38.4	34-35.9	32-33.9	30-31.9	≤29.0
Mean arterial pressure (mm Hg)	≥160	130-159	110-129		70-109		50-69		≤49
Heart rate-ventricular response	≥180	140-179	110-139		70-109		55-69	40-54	≤39
Respiratory rate per minute-non-ventilated or ventilated	≥50	35-490		25-34	12-24	10-11	6-9		≤5
Oxygen: A-a DO ₂ or PaO ₂ (Torr)									
FiO ₂ ≥0.5 record A-a DO ₂	≥500	350-499	200-349		≤200	PO ₂ 61-70		PO ₂ 55-60	PO ₂ <55
FiO ₂ <0.5 record only PaO ₂					PO ₂ >70				
Arterial pH	≥7.7	7.6-7.69		7.5-7.59	7.33-7.49		7.25-7.32	7.15-7.24	<7.15
Serum HCO ₃ (mmol/L)-only if no ABGs	≥52	41-51.9		32-40.9	23-31.9		18-21.9	15-17.9	<15
Serum sodium (mmol/L)	≥180	160-179	155-159	150-154	130-149		120-129	111-119	≤110
Serum potassium (mmol/L)	≥7	6-6.9		5.5-5.9	3.5-5.4	3-3.4	2.5-2.9		≤2.5
Serum creatinine (μmol/L)	≥350	200-340	150-190		60-140		<60		
Hematocrit (%)	≥60		50-50.9	46-49.9	30-45.9		20-29.9		≤20
White blood cell count (× 1,000/mm ³)	≥40		20-39.9	15-19.9	3-14.9		1-2.9		<1
Glasgow coma score= 15 minus actual GCS									
B: Age points			C: Chronic health points				Apache II score		
Age (years)	Points	History			Points for elective surgery	Points for emergency surgery		Sum of A+B+C	
≤44	0	Liver: Biopsy-proven cirrhosis and documented portal hypertension or prior episodes of hepatic failure			2	5		A: APS	
45-54	2	Cardiovascular: NYHA Class IV			2	5		B: Age points	
55-64	3	Respiratory: e.g., severe COPD, hypercapnia, home O ₂ , pulmonary hypertension			2	5		score	
65-74	5	Immunocompromised			2	5		C: Chronic health	
≥75	6	Renal: Chronic dialysis			2	5		point score	
Total score									

APACHE: Acute physiology and chronic health evaluation; A-a DO₂: Alveolar-arterial oxygen tension difference; PaO₂ (Torr) arterial oxygen tension; FiO₂ (%): Fractional concentration of inspired oxygen; HCO₃: Bicarbonate; ABG: Arterial blood gas; NYHA: New York heart association; COPD: Chronic obstructive pulmonary disease. To compute predicted death rates for groups of acutely ill patients, the individual risk of hospital death is calculated with the following equation; the individual risks are then summed up and the value is divided by the total number of patients. $R/1-R = -3.517 + (\text{APACHE II score} \times 0.146) + (0.603, \text{ only if post-emergency surgery}) + (\text{diagnostic category weight as shown below})$, where R is the estimated risk of hospital death

10.3 TABLE TO DERIVE $\text{PAO}_2/\text{FIO}_2$ RATIO FROM SPO_2

The $\text{PaO}_2/\text{FiO}_2$ ratio will be inferred from SpO_2 , if arterial blood gases (ABG) are unavailable, based on the following table [[R20-1709](#)]:

Measured SpO_2 (%)	Imputed PaO_2 (mmHg)
100*	167*
99*	134*
98*	104*
97*	91*
96	82
95	76
94	71
93	67
92	64
91	61
90	59
89	57
88	55
87	53
86	51
85	50
84	49
83	47
82	46
81	45
80	44
79	43

Measured SpO ₂ (%)	Imputed PaO ₂ (mmHg)
78	42
77	42
76	41
75	40
74	39
73	39
72	38
71	37
70	37
*Generally considered unreliable on the basis of the sigmoidal shape of the hemoglobin-oxygen dissociation curve. §Based on SpO ₂ 99.5%.	

10.4 POTENTIAL MODIFICATION OF TRIAL CONDUCT IN CASE OF RESTRICTIONS DUE TO COVID-19

Due to restrictions during the COVID-19 pandemic, study conduct may need to be adjusted.

In exceptional cases, when it is impossible to conduct the visits for discharged patients at the trial site, visits may be performed at the patient's home or remotely (via telephone and/or internet based means of communication). The visits may also be performed as a combination of home and remote visits. Based on the benefit-risk assessment (see [Section 1.4.1](#)), the visit procedures may be adjusted for the purpose of particular visits, whereby critical safety measures will remain in place. All home/remote visits need to be discussed with and approved by the sponsor's trial team. Local regulatory and legal requirements of the participating country need to be respected for all modifications.

Under these circumstances, the following modifications can be considered:

Remote visit

If a patient is not able to come to the site for an outpatient visit, a remote visit (by phone) should be performed instead and all assessments that can be done by phone should be performed.

Assessments that can be performed during a remote visit are:

Collection of adverse events, concomitant therapy, local laboratory. If home visits by trial staff members are possible, further assessments can be done.

Safety lab, other laboratory tests

Blood analysis for safety laboratory can be done in a local laboratory outside the hospital. The results of the laboratory tests are to be reported and transferred to the investigator, who has to ensure medical review and proper documentation in the eCRF.

Monitoring

Due to Covid-19 restrictions, on-site monitoring at participating sites may not be possible during the entire length of the trial. Therefore, on-site monitoring and on-site source data verification as foreseen in ICH GCP and noted in Boehringer-Ingelheim (BI) standard study procedures (SOPs) may not be entirely possible in this trial. If allowed at participating countries, alternate remote source data verification methods may be used, as per local laws and regulations, such as:

- Provide the monitor, under the responsibility of the investigator, with copies of the source documents/source data in which personal identifying information of the trial subjects and information pertaining to their privacy has been obscured or redacted (hereinafter referred to simply as "redacted copies").
- Grant the monitor, under the responsibility of the investigator, passive access to the source documents/source data via live image transmission (e.g. sharing of the

screen or image/sound transmission) or controlled remote access to the systems with which the source documents/source data are managed

- Arrange a facility for the on-site monitoring with access to patient files / Investigator Site File not subject to Covid-19 ward.
- Arrange a verified remote SDV platform that allows upload of obscured or redacted trial source documents by sites, and remote review by CRA.

10.5 ALTEPLASE DOSING TABLE

Dosing regimen A (lower dose) dosing table

TRISTARDS trial DOSING TABLE for Alteplase (Dosing regimen A - lower dose) (1mg/mL concentration)				
Weight (kg)*	Short-term infusion over 2 hours (Day 1 only / or optional re-application)		Long-term infusion over 12 hours (Daily from Day 1 up to day 5)	
	Infusion Dose over 2 hours (mg) (0,3 mg/kg)	Infusion Rate (mL/hour)	Infusion Dose over 12 hours (mg) (0,02 mg/kg/h)	Infusion Rate (mL/hour)
40	12.0	6.0	9.6	0.8
42	12.6	6.3	10.1	0.8
44	13.2	6.6	10.6	0.9
46	13.8	6.9	11.0	0.9
48	14.4	7.2	11.5	1.0
50	15.0	7.5	12.0	1.0
52	15.6	7.8	12.5	1.0
54	16.2	8.1	13.0	1.1
56	16.8	8.4	13.4	1.1
58	17.4	8.7	13.9	1.2
60	18.0	9.0	14.4	1.2
62	18.6	9.3	14.9	1.2
64	19.2	9.6	15.4	1.3
66	19.8	9.9	15.8	1.3
68	20.4	10.2	16.3	1.4
70	21.0	10.5	16.8	1.4
72	21.6	10.8	17.3	1.4
74	22.2	11.1	17.8	1.5
76	22.8	11.4	18.2	1.5
78	23.4	11.7	18.7	1.6
80	24.0	12.0	19.2	1.6
82	24.6	12.3	19.7	1.6
84	25.2	12.6	20.2	1.7
86	25.8	12.9	20.6	1.7
88	26.4	13.2	21.1	1.8
90	27.0	13.5	21.6	1.8
92	27.6	13.8	22.1	1.8
94	28.2	14.1	22.6	1.9
96	28.8	14.4	23.0	1.9

TRISTARDS trial DOSING TABLE for Alteplase (Dosing regimen A - lower dose) (1mg/mL concentration)				
Weight (kg)*	Short-term infusion over 2 hours (Day 1 only / or optional re- application)		Long-term infusion over 12 hours (Daily from Day 1 up to day 5)	
	Infusion Dose over 2 hours (mg) (0,3 mg/kg)	Infusion Rate (mL/hour)	Infusion Dose over 12 hours (mg) (0,02 mg/kg/h)	Infusion Rate (mL/hour)
98	29.4	14.7	23.5	2.0
100	30.0	15.0	24.0	2.0
102	30.6	15.3	24.5	2.0
104	31.2	15.6	25.0	2.1
106	31.8	15.9	25.4	2.1
108	32.4	16.2	25.9	2.2
110	33.0	16.5	26.4	2.2
112	33.6	16.8	26.9	2.2
114	34.2	17.1	27.4	2.3
116	34.8	17.4	27.8	2.3
118	35.4	17.7	28.3	2.4
≥120	36.0	18.0	28.8	2.4

*actual weight is preferred

(estimated weight is acceptable, if actual weight is not available).

Patients which body weight is in between two categories (e.g. 51 kg) should be administered the dosage of the next higher dosing category (e.g. 52 kg).

Dosing regimen B (higher dose) dosing table

TRISTARDS trial DOSING TABLE for Alteplase (Dosing regimen B - higher dose) (1mg/mL concentration)				
Weight (kg)*	Short-term infusion over 2 hours (Day 1 only / or optional re- application)		Long-term infusion over 12 hours (Daily from Day 1 up to day 5)	
	Infusion Dose over 2 hours (mg) (0,6 mg/kg)	Infusion Rate (mL/hour)	Infusion Dose over 12 hours (mg) (0,04 mg/kg/h)	Infusion Rate (mL/hour)
40	24.0	12.0	19.2	1.6
42	25.2	12.6	20.2	1.7
44	26.4	13.2	21.1	1.8
46	27.6	13.8	22.1	1.8
48	28.8	14.4	23.0	1.9
50	30.0	15.0	24.0	2.0
52	31.2	15.6	25.0	2.1
54	32.4	16.2	25.9	2.2
56	33.6	16.8	26.9	2.2
58	34.8	17.4	27.8	2.3
60	36.0	18.0	28.8	2.4
62	37.2	18.6	29.8	2.5
64	38.4	19.2	30.7	2.6
66	39.6	19.8	31.7	2.6
68	40.8	20.4	32.6	2.7
70	42.0	21.0	33.6	2.8
72	43.2	21.6	34.6	2.9
74	44.4	22.2	35.5	3.0
76	45.6	22.8	36.5	3.0
78	46.8	23.4	37.4	3.1
80	48.0	24.0	38.4	3.2
82	49.2	24.6	39.4	3.3
84	50.4	25.2	40.3	3.4
86	51.6	25.8	41.3	3.4
88	52.8	26.4	42.2	3.5
90	54.0	27.0	43.2	3.6
92	55.2	27.6	44.2	3.7
94	56.4	28.2	45.1	3.8
96	57.6	28.8	46.1	3.8
98	58.8	29.4	47.0	3.9
100	60.0	30.0	48.0	4.0

TRISTARDS trial DOSING TABLE for Alteplase (Dosing regimen B - higher dose) (1mg/mL concentration)				
Weight (kg)*	Short-term infusion over 2 hours (Day 1 only / or optional re- application)		Long-term infusion over 12 hours (Daily from Day 1 up to day 5)	
	Infusion Dose over 2 hours (mg) (0,6 mg/kg)	Infusion Rate (mL/hour)	Infusion Dose over 12 hours (mg) (0,04 mg/kg/h)	Infusion Rate (mL/hour)
102	61.2	30.6	49.0	4.1
104	62.4	31.2	49.9	4.2
106	63.6	31.8	50.9	4.2
108	64.8	32.4	51.8	4.3
110	66.0	33.0	52.8	4.4
112	67.2	33.6	53.8	4.5
114	68.4	34.2	54.7	4.6
116	69.6	34.8	55.7	4.6
118	70.8	35.4	56.6	4.7
≥120	72.0	36.0	57.6	4.8
<p>*actual weight is preferred (estimated weight is acceptable, if actual weight is not available).</p> <p>Patients which body weight is in between two categories (e.g. 51 kg) should be administered the dosage of the next higher dosing category (e.g. 52 kg).</p>				

11. DESCRIPTION OF GLOBAL AMENDMENT(S)

11.1 GLOBAL AMENDMENT 1

Date of amendment		22 Oct 2020
EudraCT number EU number		2020-002913-16
BI Trial number		0135-0347
BI Investigational Product(s)		Alteplase (recombinant tissue-type plasminogen activator, rt-PA)
Title of protocol		<p>The TRISTARDS trial - ThRombolysisIS Therapy for ARDS</p> <p>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.</p>
To be implemented only after approval of the IRB / IEC / Competent Authorities		x
To be implemented immediately in order to eliminate hazard – IRB / IEC / Competent Authority to be notified of change with request for approval		
Can be implemented without IRB / IEC / Competent Authority approval as changes involve logistical or administrative aspects only		
Section to be changed		Flowchart
Description of change 1		<p>Vital signs and laboratory tests added at Day 6 IRT call and drug dispensation at Day 2,3 and 4</p> <p><i>changed to:</i></p> <p>removed</p>
Rationale for change		<p>Mistakenly entered for IRT call; vital signs and laboratory tests added at day 6 because these are required for SOFA and APACHE II score calculation.</p>

Section to be changed		Flowchart , Footnote 6
Description of change 2		<p>Laboratory samples - haematology, clinical chemistry, renal function and coagulation parameters including D-dimer and fibrinogen are collected at screening, on Days 3, 5, 7 and 28.</p> <p><i>changed to:</i></p> <p>On Day 6 only lab samples required for APACHE II, SOFA score and PaO₂/FiO₂ shall be collected.</p>
Rationale for change		Wording in Footnote 6 was splitted and separated for lab tests supporting scores calculation and D-dimer and other coagulation parameters assessments for more clarity
Section to be changed		Flowchart, Footnote 10
Description of change 3		<p>APACHE II score will be evaluated up to Day 28 and SOFA will be assessed until Day 6</p> <p><i>changed to:</i></p> <ol style="list-style-type: none"> 1. APACHE II score will be evaluated up to Day 28 and SOFA will be assessed until Day 6 including vital signs assessment and laboratory samples needed for score calculations. <u>If patient is discharged from hospital prematurely, last evaluation during hospital stay is expected at day of discharge from hospital</u>
Rationale for change		Details on procedures needed to support score calculation
Section to be changed		Section 1.4.2
Description of change 4		Added: <u>Effect of Alteplase on D-Dimer and fibrinogen</u>
Rationale for change		Increased safety, guidance for physicians for changes in coagulation system
Section to be changed		Section 3.3.1 Inclusion criteria #2
Description of change 5		<p>ARDS with PaO₂*/FiO₂ ratio >100 and ≤300 , either on non-invasive ventilator support, OR on mechanical ventilation (<48 hours since intubation),</p> <ul style="list-style-type: none"> - with bilateral opacities in chest X-ray and

		CT scan (not fully explained by effusions, lobar/lung collapse, or nodules) - <i>changed to:</i> - with bilateral opacities in chest X-ray or CT scan (not fully explained by effusions, lobar/lung collapse, or nodules)
Rationale for change		Typo correction
Section to be changed		Section 5.2.6.2.2
Description of change 6		The investigator must report serious adverse events, and non-serious adverse events which are relevant for the reported SAE on the BI SAE form via fax <i>Changed to:</i> The investigator must report serious adverse events, and non-serious adverse events which are relevant for the reported SAE on the BI SAE form via fax (except of Russia and UK where SAE form is to be send via Clinergize)
Rationale for change		Reflecting current SAE reporting process flow
Section to be changed		Section 5.3.2
Description of change 7		Blood for quantification of analyte plasma concentrations will be withdrawn either by means of indwelling venous catheter or venipuncture <i>Changed to:</i> Blood for quantification of analyte plasma concentrations will be withdrawn by single-time venipuncture
Rationale for change		Refinement of pre-analytical phase
Section to be changed		Appendix 10.2
Description of change 8		SOFA score table was replaced with new one and reference to calculator for SOFA score removed
Rationale for change		Ensure unique score calculation as referenced
Section to be changed		Section 8.3.1
Description of change 9		Covid-10 <i>Changed to:</i> Covid-19

Rationale for change		Typo correction
-----------------------------	--	-----------------

APPROVAL / SIGNATURE PAGE

Document Number: c32434345

Technical Version Number: 2.0

Document Name: clinical-trial-protocol-version-02

Title: The TRISTARDS trial - Thrombolysis Therapy 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.

Signatures (obtained electronically)

Meaning of Signature	Signed by	Date Signed
Approval-Therapeutic Area Head	Brueckmann, Prof. Dr., Martina	22 Oct 2020 13:50 CEST
Approval-Clinical Trial Leader	Hollaenderova, Dr., Zuzana	22 Oct 2020 14:19 CEST
Author-Clinical Pharmacokineticist	Glund, Dr., Stephan	22 Oct 2020 18:54 CEST
Approval-Team Member Medical Affairs	Pages, Dr., Alain	22 Oct 2020 19:54 CEST
Approval-Team Member Medicine	Gergei, Dr., Ingrid	23 Oct 2020 13:04 CEST
Approval	Peter, Nuala	23 Oct 2020 13:58 CEST
Verification-Paper Signature Completion	Hollaenderova, Dr., Zuzana	23 Oct 2020 14:27 CEST

(Continued) Signatures (obtained electronically)

Meaning of Signature	Signed by	Date Signed
----------------------	-----------	-------------