Clinical Trial Protocol

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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 - **Th**ro**mbolysIS T**herapy for **ARDS** A Phase IIb/III operationally seamless, open-label, randomised, sequential, parallel-group adaptive study to evaluate the efficacy and safety of daily intravenous alteplase treatment given up to 5 days on top of standard of care (SOC) compared with SOC alone, in patients with acute respiratory distress syndrome (ARDS) triggered by COVID-19.	
Lay Title	The **TRISTARDS** trial - **Th**ro**mbolysIS T**herapy 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	[redacted] Phone: [redacted] Fax: [redacted]	
Coordinating Investigator	[redacted] Tel. [redacted] Fax. [redacted]	
Version and Date	**Version: 4.0**	**Date: 17 Feb 2022**

CLINICAL TRIAL PROTOCOL SYNOPSIS

Company name	Boehringer Ingelheim (BI)
Protocol date	21 July 2020
Revision date	17 Feb 2022
BI trial number	0135-0347
Title of trial	The **TRISTARDS** trial - **T**h**R**ombolys**IS** **T**herapy for **ARDS** A Phase IIb/III operationally seamless, open-label, randomised, sequential, parallel-group adaptive study to evaluate the efficacy and safety of daily intravenous alteplase treatment given up to 5 days in addition to standard of care (SOC) compared with SOC alone, in patients with acute respiratory distress syndrome (ARDS) triggered by COVID-19.
Coordinating Investigator	[redacted] Tel. [redacted] Fax
Trial site(s)	Multi-centre trial conducted in approximately 22 countries
Clinical phase	IIb/III
Trial rationale	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.
Trial objective(s)	To evaluate the efficacy and safety of intravenous alteplase in ARDS triggered by COVID-19.
Trial endpoints	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. In Part 2, key secondary endpoints are: • Treatment failure defined as all cause mortality or mechanical ventilation at Day 28 • All cause mortality at Day 28 Secondary endpoints to evaluate safety and efficacy in Part 2 are as follows: • Number of oxygen-free days up to Day 28 • Length of hospital stay up to Day 28 • Major bleeding events (MBE) (according to International Society on Thrombosis and Haemostasis [ISTH] definition) until Day 6 • PaO_2/FiO_2 ratio (or inferred PaO_2/FiO_2 ratio from SpO_2) change from baseline to Day 6
Trial design	Open-label, randomised, sequential parallel design comparison of: Part 1: two treatment arms and a standard of care (SOC) group (1:1:1 randomisation) Part 2: one treatment arm and a standard of care (SOC) group (2:1 randomisation)
Total number of patients randomised	Part 1: approximately 60 patients Part 2: approximately 260 patients, thereof 210 non-invasive mechanical ventilation (NIV) and up to 50 invasive mechanical ventilation (IMV) patients Randomisation will be stratified by type of ventilation support (invasive mechanical / non-invasive) Additionally in Part 2: Randomisation will be stratified by D-Dimer levels (≥ULN to <5-fold ULN, versus ≥5-fold ULN) Randomisation of IMV patients will be stopped when a maximum of 50 IMV patients have been randomized in Part 2. Randomisation for the whole trial will be stopped when approximately 210 NIV patients have been randomized in Part 2, regardless of how many IMV patients have been randomised so far.
Number of patients on each treatment	Part 1: approximately 20 patients in SOC arm and 20 patients in each treatment arm Part 2: NIV patients: approximately 70 patients in SOC arm and 140 in treatment arm

	IMV patients: up to approximately16 patients in SOC arm and 32 in treatment arm
Diagnosis	ARDS due to COVID-19
Main in- and exclusion criteria	Patients participating in Part 1 are not eligible for Part 2. **Main criteria for inclusion:** 1. Age ≥ 18 years (or above legal age) 2. ARDS with PaO_2*/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 PaO_2/FiO_2 from pulse oximetry (SpO_2/FiO_2) 3. SARS-CoV-2 positive (laboratory-confirmed RT-PCR test) 4. Fibrinogen level ≥ lower limit of normal 5. D-Dimer ≥ 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. **Main criteria for exclusion:** 1. Massive confirmed pulmonary embolism (PE) with haemodynamic instability at trial entry, or any (suspected or confirmed) PE that is expected to require therapeutic dosages of anticoagulants during the treatment period 2. Indication for therapeutic dosages of anticoagulants at trial entry 3. Mechanical ventilation for longer than 48 hours 4. Chronic pulmonary disease i.e. with known forced expiratory volume in 1 second (FEV_1) <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 5. Has a Do-Not-Intubate (DNI) or Do-Not-Resuscitate (DNR) order 6. In the opinion of the investigator, is not expected to survive for > 48 hours after screening. 7. Planned interventions during the first 5 days after randomisation, such as surgery, insertion of central catheter or arterial line, drains, etc. Further criteria apply to exclude patients at higher risk of bleeding.

Test product(s)	Alteplase, recombinant tissue-plasminogen activator, rt- PA, powder and solvent for infusion
dose	Part 1: 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) *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)* OR: 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) *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)* Part 2: 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) *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)* ** Exception: Treatment period can be exceeded beyond Day 5, in case of unavoidable interruptions of the treatment.*
mode of administration	Intravenous (i.v.)
Comparator product(s)	N/A
dose	N/A
mode of administration	N/A
Duration of treatment	Maximum of 5 days. In case of unavoidable and urgent reasons, treatment interruptions are permitted up to a total of 72 hrs, and the treatment period will be extended accordingly.

Statistical methods	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. 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. 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. Part 2 will consist of two cohorts, the NIV patient cohort and the IMV patient cohort, and these two cohorts will be analysed separately. Only the NIV patient cohort will be part of the main statistical objectives of the study, and therefore part of the confirmatory hierarchical testing procedure. In Part 2, a confirmatory hierarchical testing procedure in the NIV patient cohort will be applied for the primary endpoint and the two key secondary endpoints. In a first step, the primary endpoint hypothesis in NIV patients will be tested. If the null hypothesis is rejected, the key secondary endpoint of Treatment failure at Day 28 will then be tested in the NIV patient cohort as a second step. If this null hypothesis is rejected, the key secondary endpoint All cause mortality at Day 28 will then be tested in the NIV patient cohort as a third step. 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 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. For the primary endpoint the Cox proportional hazard model will be used to estimate the hazard ratio, the 95% confidence interval and p-value. In the NIV cohort, adjustment will be made for the number of days under NIV support, baseline D-Dimer level and age. For the

	IMV cohort, adjustment will be made for the baseline WHO value, baseline D-Dimer level and age. For each of the two key secondary endpoints, treatment failure at Day 28 and all cause mortality at Day 28, the delta method and average marginal effect method will be used to calculate the risk difference, 95% confidence intervals and p-values. Adjustment will be made for the same set of covariates as in the primary analysis of the primary endpoint. Descriptive statistics will be used to analyse all other endpoints and for all analyses of the IMV patient cohort. If the NIV and IMV patient cohorts show homogeneity, then a meta-analysis will be performed to obtain an overall treatment estimate, albeit in an exploratory manner.

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								
Administer trial drug[4]		X	X	X	X	X				
PK Sampling[5]		X								
Laboratory Tests[6]	X			X		X	X[6]		X	
D-dimer assessment[7]	X			X		X		X	X	
Fibrinogen, aPTT and antithrombin assessment[8]	X	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	
[redacted]										
Trial specific examinations: SOFA score[10]	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									
All adverse events/Serious adverse events[14]	X	X	X	X	X	X	X	X	X	
Concomitant therapy	X	X	X	X	X	X	X	X	X	
Completion of patient participation[15]										X
Vital status collection[16]										X

.Footnotes:

1. Vital signs measurement shall include BP measurement, body temperature, respiratory rate and pulse rate.
2. 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).
3. Randomisation of patients shall happen not later than 24 hours after the confirmation of the inclusion and exclusion criteria. Study drug administration shall start within 6 hours of the randomisation. The time-point of obtaining D-Dimer levels can be as long as four calendar days before screening.
4. In Part 2: 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, plus possible interruption time for urgent reasons). Interruptions up to 72 hours in total for urgent reasons are allowed and accordingly, the time window of the visits may be extended. The very first infusion of alteplase should start at least 10 hours after the last injection of a LMWH. Each daily 12-hour infusion of alteplase is followed by an interruption of approximately 12 hours. Does not apply for SOC treatment arm.
5. PK samples collection (on a voluntary basis, 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.
6. On Day 6 only lab samples required for SOFA score and PaO_2/FiO_2 shall be collected.
7. D-dimer levels shall be measured at Day 0 (or no more than 4 calendar days before screening), days 3, 5, 7 and 28
8. During the treatment period, fibrinogen should be measured daily. The aPTT should be measured at least daily, but preferably more often. The aPTT (for all patients on unfractionated heparin [UFH]) or anti-Xa (for all patients on low-molecular weight heparin [LMWH]) should be used to check the intensity of anticoagulation. In patients with moderate (creatinine clearance 30-50 ml/min) and mild (creatinine clearance 50-80 ml/min) renal impairment, careful clinical monitoring of anticoagulation intensity according to institutional guidance is advised. (more details see Section 4.2.1)
9. PaO_2/FiO_2 is only measured during hospital stay. The qualifying PaO_2/FiO_2 ratio should be the worst measurement in the screening period. During the treatment period the worst measurement on a given day should be documented in the case report form. During the first 6 days and during the stay on ICU (whatever is longer), PaO_2/FiO_2 ratio should be measured at least three times daily in all patients, together with a recording of the patient`s position.
10. The SOFA score will be assessed at baseline, Day 6 and Day 28 (or just before hospital discharge whichever comes first including vital signs assessment and laboratory samples needed for score calculations. (Please refer to Appendix 10.1). If patient is discharged from hospital prematurely, last evaluation during hospital stay is expected at day of discharge from hospital.
11. WHO clinical progression scale should be collected daily during hospital stay. The last measurement on a given day should be recorded in the case report form. On Day 1 (randomisation day) an assessment just before randomisation shall be collected as a baseline value.
12. 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. A woman is considered of childbearing potential, i.e. fertile, following menarche and until becoming post-menopausal unless permanently sterile. Permanent sterilisation methods include hysterectomy, bilateral salpingectomy and bilateral oophorectomy. Tubal ligation is NOT a method of permanent sterilisation. A postmenopausal state is defined as no menses for 12 months without an alternative medical cause.
13. SARS-CoV-2 laboratory confirmation (RT-PCR test) as a pre-requisite before or at screening. The RT-PCR test needs to be in timely association with the current hospital stay. For eligibility, patients should be hospitalized with the diagnosis of COVID-19 ARDS. The choice of laboratory tests should follow local practice.

14. The rules for Adverse Event Reporting exemptions still apply, please see Section 5.2.6.2.4.
15. Patients who discontinue trial treatment prematurely should still follow the trial schedule until Visit 6/EOS, if possible, please see Section 3.3.4.
16. 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.

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ABBREVIATIONS

ACE	angiotensin-converting enzyme
AE	adverse event
ALCOA	attributable, legible, contemporaneous, original, accurate
ALT	alanine aminotransferase
AME	Average marginal effect
[redacted]	[redacted]
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
IMV	Invasive mechanical ventilation
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
NIV	Non-invasive mechanical ventilation
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
T2DM	Type 2 diabetes mellitus
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.

Since the outbreak of COVID-19, treatment options for hospitalized patients with COVID-19 who require oxygen and ventilation support are evolving. Data and guidance to target the

hyperinflammatory status of COVID-19 patients are available [R21-2944, R20-4049, R21-2943, R21-2942, R21-2936]. [R20-1647, R21-2937, R21-2938, R20-1645, R21-2941].

With regard to the need and intensity of concomitant anticoagulation, the evidence rapidly evolved during the SARS-CoV-2 pandemic. Guidelines recommend prophylactic anticoagulation for all patients with COVID-19 in the ICU and do not recommend therapeutic anticoagulation in absence of known thromboembolic events [R20-1852, R21-2851]. The status of intermediate dosing of anticoagulants in critically ill patients is still under scientific debate [P21-01227, R21-2935].

Critically ill COVID-19 patients with hypercoagulable state, presenting with a significant increase in D-dimer levels, might benefit from a thrombolytic therapy on top of anticoagulation with unfractionated or low-molecular weight heparins. 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 pharmaceutic 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.

Results of phase II (Part 1)

Efficacy
Observed data from Phase II (Part 1), in line with the dose-finding objective, demonstrated the alteplase 0.6/0.04 mg/kg dose in addition to SOC was associated with faster clinical improvement or hospital discharge, compared with the alteplase 0.3/0.02 mg/kg + SOC, or SOC alone treatments.

Data from patients in the alteplase 0.6/0.04 mg/kg group show numerically more favourable outcomes of clinical improvement of ≥2 points on the 11-point WHO Clinical Progression

Scale or hospital discharge by Day 28 than those in the SOC group (adjusted HR 2.04 [95% CI 0.83 to 5.01], p=0.1183). The alteplase 0.6/0.04 mg/kg group had a median time of 19 days to clinical improvement or hospital discharge; the median was not achieved in the alteplase 0.3/0.02 mg/kg or the SOC groups. No difference on the primary endpoint was observed between the alteplase 0.3/0.02 mg/kg and the SOC groups.

The favorable results on the primary endpoint in the alteplase 0.6/0.04 mg/kg dosing group were supported by similar trends for other prespecified analyses, including sensitivity analyses of the primary endpoint, as well as secondary efficacy endpoints of lower all-cause mortality and lower all-cause mortality or mechanical ventilation up to Day 28 compared with the SOC group, greater improvement in median PaO2/FiO2 ratio from baseline to Day 6 and more ventilator-free days from the start of treatment to Day 28 than in the alteplase 0.3/0.02 mg/kg and SOC groups.

A Kaplan-Meier estimate of the unadjusted primary analysis showed a separation of the alteplase 0.6/0.04 mg/kg group compared with the other two treatment groups, beginning at approximately Day 11. The risk difference for the alteplase 0.6/0.04 mg/kg group compared with the SOC group at Day 28 was 19.1% in favour of alteplase (95% CI -10.6%, 48.8%).

Secondary and further endpoint results supported the clinical benefit of alteplase in addition to SOC, particularly for the alteplase 0.6/0.04 mg/kg group. Both alteplase groups had numerically fewer cases of all-cause mortality than in the SOC group at Day 28. All-cause mortality or mechanical ventilation at Day 28 was considered as treatment failure; both alteplase groups had fewer cases of all-cause mortality or mechanical ventilation at Day 28 than the SOC group. The alteplase 0.6/0.04 mg/kg group had more ventilator-free days from the start of treatment to Day 28 than the alteplase 0.3/0.02 mg/kg and SOC groups. In addition, the alteplase 0.6/0.04 mg/kg group had a greater improvement in median PaO_2/FiO_2 ratio from baseline to Day 6 compared with the alteplase 0.3/0.02 mg/kg or SOC group..

[redacted]

Safety
The overall safety results were generally comparable across the treatment groups. Patients treated with alteplase and those receiving only standard of care had similar frequencies of any adverse events (AEs), severe AEs, and serious AEs. The majority of reported AEs were of mild or moderate intensity. No new safety signals were identified.

The most common AEs by system organ class were: respiratory, thoracic and mediastinal disorders (alteplase 0.3/0.02 mg/kg: 7 patients/35.0%, alteplase 0.6/0.04 mg/kg: 12 patients/60.0%, SOC: 9 patients/40.9%), infections and infestations (9 patients/45.0%, 4 patients/20.0%, 11 patients/50.0%), and general disorders and administration site conditions (7 patients/35.0%, 7 patients/35.0%, 4 patients/18.2%).

Serious AEs reported for >2 patients in a treatment group were respiratory failure (alteplase 0.3/0.02 mg/kg: 2 patients/10.0%, alteplase 0.6/0.04 mg/kg: 0 patients, SOC: 6 patients/27.3%), pulmonary embolism (2 patients/10.0%, 3 patients/15.0%, 1 patient/4.5%), septic shock (1 patient/5.0%, 0 patients, 4 patients/18.2%), and epistaxis (3 patients/15.0%, 1 patient/5.0%, 0 patients).

Treatment-emergent bleeding events were more frequent in the alteplase treatment groups, both for any bleeding events and major bleeding events. Major bleeding events up to Day 6 were reported only in the alteplase treatment groups (1 patient in the 0.3/0.02 mg/kg dosing group and 4 patients in the 0.6/0.04 mg/kg dosing group). Most bleeding events until Day 12 were non-major (4 patients, alteplase 0.3/0.02 mg/kg; 9 patients, alteplase 0.6/0.04 mg/kg; 3 patients, SOC group). There were no fatal bleeding events.

The study results of Part 1 support the continuation of the study into Phase III (Part 2), implementing a comparison of the alteplase 0.6/0.04 mg/kg dose versus SOC alone.

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 w
as 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 likely 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 with haemodynamic instability and acute ischemic stroke.

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 acute ischemic 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 study results of Phase II of the current trial (Part 1) provided proof of concept and support the continuation of the study into Phase III (Part 2), the confirmatory part of the study, implementing a comparison of the alteplase 0.6/0.04 mg/kg dose versus SOC alone.

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 in both parts of the trial (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 thrombogenic 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 LMWH 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. The results of Phase II of the current trial showed that patients receiving a dose of 0.6 mg/kg over 2 hours immediately followed by a constant rate infusion of alteplase 0.04 mg/kg/h over 12 hours (up to 5 days) in addition to SOC was associated with faster clinical improvement or hospital discharge, compared with patients receiving alteplase 0.3/0.02 mg/kg on top of SOC, or SOC alone. The study results of Phase II (Part 1), support the continuation of the study into Phase III (Part 2), the confimartory part of the study.
Alteplase can be given in combination with UFH or LMWH. This is routinely performed when applying treatment to acute MI patients or acute PE patients.

During the alteplase treatment period of up to a maximum of 5 days (plus a possible extension in case of unavoidable interruptions of up to 72 hours) the concomitant use of LMWH in Part 2 will be limited to prophylactic doses and the use of UFH will be limited to target levels of aPTT between 1.0 to 1.5 fold ULN according to local laboratory.

Treating patients with a moderate initial dose of 0.6 mg/kg over 2 hours (for example, this corresponds to 42 mg in a patient with 70 kg of body weight) combined with a constant rate infusion (CRI) of low dose alteplase of 0.04 mg/kg/h over 12 hours is considered to reduce the risk of alteplase-related bleeding events in comparison to application of high alteplase doses (up to 100 mg) used in other indications (e.g. massive pulmonary embolism with hemodynamic instability, acute ischemic stroke, acute myocardial infarction). Also a regular temporary interruption of 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 including i.v. tranexamic acid may be considered.

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 PaO_2/FiO_2 ratio) of COVID-19 related ARDS.

COVID-19 ARDS 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

60 minutes were symptomatic intracerebral haemorrhage (6%) and other major haemorrhage (2%). Minor haemorrhage (e.g. bleeding at site of venepuncture) accounting for 20-25%.

Similarly, in patients with acute MI where alteplase doses (up to 100 mg) given over 90 min 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].

In the Phase II part of the current trial, no new safety signals were identified. Major bleeding events until Day 6 were overall infrequent (1 patient in the 0.3/0.02 mg/kg dosing group and 3 patients in the 0.6/0.04 mg/kg dosing group), and a trend towards a dose-dependent increase was mainly observed for non-major bleeding events.
Mitigation strategies to prevent bleeding events in Phase III (Part 2) of the current study are implemented in Section 3.3.4.1.

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

Table 1.4.2:1 Overview of trial-related risks (cont)

Clinical events		
Minor bleeding particularly traumatic –at site of venepuncture		Supportive treatment, and use caution during suction, measurement of plasma fibrinogen levels
Major bleeding including intracerebral haemorrhage or bleeding from a critical organ	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 should be considered
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 patient, 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 antithrombotics. 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 safety results from Phase II (part 1) of the current trial were generally comparable across the treatment groups, except for bleeding events, which are a known side effect of alteplase. In Phase II, mainly non-major bleeding events were observed, and some of them were related to interventions. Additional risk minimization strategies to avoid intervention-related bleeding events have accordingly been implemented for Part 2, phase III of the study.

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, alteplase has to be temporarily interrupted. 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. The study results of Phase II (Part 1), support the continuation of the study into Phase III (Part 2), the confirmatory part of the study. The alteplase 0.6/0.04 mg/kg dose in addition to SOC was associated with faster clinical improvement [redacted], compared with the

alteplase 0.3/0.02 mg/kg + SOC, or SOC alone treatments. [redacted]. The safety results were generally comparable across the treatment groups. No new safety signals were identified. Major bleeding events were overall infrequent, and a trend towards a dose-dependent increase was mainly observed for non-major bleeding events.
Precautionary recommendations for temporary interruption of alteplase infusion (up to 72 hours in total for urgent reasons, bleeding or drop in fibrinogen), a limitation of concomitant administration of heparins to prophylactic doses and recommendations to avoid intervention-related bleeding events are implemented in Part 2 to mitigate the risk of bleeding.
Taken together, the overall benefit-risk ratio of alteplase at the studied dosing regimen is considered favourable.

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 following secondary endpoints to assess safety and efficacy in this setting have been evaluated in Part 1:

- 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 PaO_2/FiO_2 ratio (or inferred PaO_2/FiO_2 ratio from SpO_2) change from baseline to Day 6
- All-cause mortality or on mechanical ventilation at Day 28

In Part 2, the following key secondary endpoints will be evaluated:

- Treatment failure defined as all cause mortality or mechanical ventilation at Day 28
- All cause mortality at Day 28

In Part 2, the following secondary endpoints will be evaluated:

- Number of oxygen-free days up to Day 28
- Length of hospital stay up to Day 28
- 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.
- PaO_2/FiO_2 ratio (or inferred PaO_2/FiO_2 ratio from SpO_2) change from baseline to Day 6

[redacted]

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 approximately 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 approximately 260 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 primary endpoint. Visit schedules are the same in both parts. There will be one interim assessment after Part 1.
One dosing regimen of alteplase (0.6/0.04 mg/kg dosing, see Section 4.1.1) has been 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.

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

Randomisation in Part 2 will be 2:1 to dosing regimen B of alteplase (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 or to standard of care, and will be stratified by type of ventilation (non-invasive or invasive mechanical) and by D-dimer levels (≥ULN to <5-fold ULN, versus ≥5-fold ULN).
Recruitment of patients on mechanical ventilation will be stopped when a maximum of 50 patients have been randomized in Part 2. Randomisation for the whole trial will be stopped when approximately 210 NIV patients have been randomized in Part 2, regardless of how many IMV patients have been randomised so far.

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):

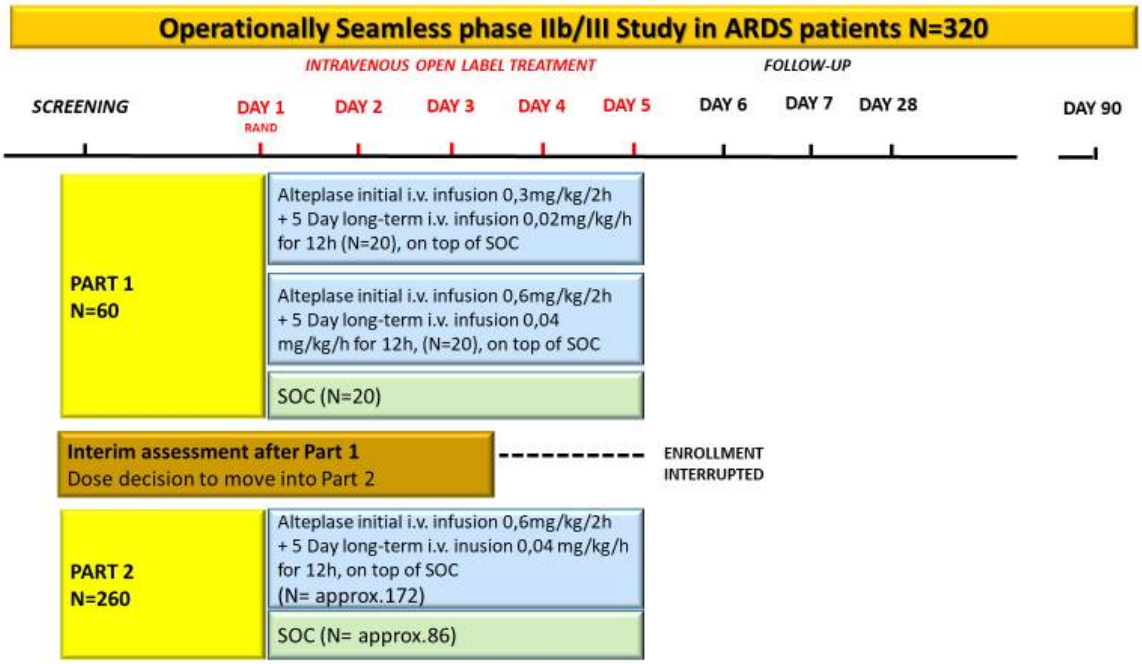

Figure 3.1:1 Study design

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. As Part 1 results indicated the need for some design modifications in Part 2 beyond those pre-specified in the protocol, these required modifications have been implemented via the current 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 has provided a recommendation to continue into Part 2, together with a recommendation to select dosing regimen B for Part 2. For further details, see the DMC Charter and the DMC recommendation after completion of Part 1.

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 260 patients planned to be randomised at approximately 110-130 sites and approximately 2-3 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 or the incidence of new COVID-19 cases shifts to other regions of the world, the trial may expand to Eastern Europe, Russia, China, India, Africa, South America and other countries to focus on places where the incidence of COVID-19 is high and vaccination rate is slow. Approximately 22 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.
In part 2 of the trial, a randomisation cap will be applied for patients on mechanical ventilation at baseline: when 50 patients on mechanical ventilation have been randomized. Recruitment of further patients on mechanical ventilation will be stopped via IRT.

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 time-point of obtaining D-Dimer levels can be as long as four calendar days before screening.

The respiratory status of the enrolled patients is monitored by PaO_2/FiO_2 ratio (or inferred PaO_2/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 qualifying PaO_2/FiO_2 ratio should be the worst measurement in the screening period. During the treatment period the worst measurement on a given day should be documented in the case report form.

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

Patients participating in Part 1 are not eligible for Part 2.

1. Age ≥ 18 years (or above legal age)
2. ARDS with PaO_2*/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 PaO_2/FiO_2 from pulse oximetry (SpO_2/FiO_2) (see Appendix 10.2 for SpO_2/FiO_2 estimation)
3. SARS-CoV-2 positive (laboratory-confirmed RT PCR test)
4. Fibrinogen level ≥ lower limit of normal (according to local laboratory)
5. D-Dimer ≥ 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, or any (suspected or confirmed) PE that is expected to require therapeutic dosages of anticoagulants during the treatment period
2. Indication for therapeutic dosages of anticoagulants at trial entry
3. Mechanical ventilation for longer than 48 hours
4. Chronic pulmonary disease i.e. with known FEV_1 <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
5. Has a Do-Not-Intubate (DNI) or Do-Not-Resuscitate (DNR) order
6. In the opinion of the investigator not expected to survive for > 48 hours after screening.
7. Planned interventions during the first 5 days after randomisation, such as surgery, insertion of central catheter or arterial line, drains, etc.
8. Known hypersensitivity to the active substance alteplase, gentamicin (a trace residue from the manufacturing process) or to any of the excipients
9. Significant bleeding disorder at present or within the past 6 months, known haemorrhagic diathesis
10. 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
11. Any history of central nervous system damage (i.e. neoplasm, aneurysm, intracranial or spinal surgery)
12. History or evidence or suspicion of intracranial haemorrhage including sub-arachnoid haemorrhage
13. Severe uncontrolled arterial hypertension (according to the investigator`s judgement)
14. Major surgery or significant trauma in the past 10 days, recent trauma to head or cranium
15. Cardiac arrest and/or cardiopulmonary resuscitation during the current hospital stay
16. Obstetrical delivery within the past 10 days
17. Severe hepatic dysfunction i.e. Child-Pugh B and C, including biopsy confirmed hepatic cirrhosis, portal hypertension, hepatic encephalopathy, or active hepatitis
18. Bacterial endocarditis, pericarditis

19. Acute pancreatitis
20. Documented ulcerative gastro-intestinal disease during the last 3 months
21. Severe heart failure (New York Heart Association Class IV)
22. Arterial aneurysms, arterial/venous malformations
23. Malignancy (Stage IV) with increased bleeding risk
24. Haemorrhagic stroke or stroke of unknown origin at any time
25. Ischaemic stroke or transient ischaemic attack (TIA) in the preceding 6 months
26. 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.
27. 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.)
28. End stage renal disease (Stage ≥ 4) or need for renal replacement therapy at baseline
29. Need for ECMO at baseline
30. Platelet count <100 x 10^9/L or history of Heparin-Induced Thrombocytopenia
31. Lumbar puncture with in past 3 days
32. Known abdominal or thoracic aneurysm
33. 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).

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 the investigator
- in case of clinical improvement of respiratory situation with sustained (as per investigator`s judgement) PaO_2/FiO_2 ratio above 300.
- Need for ECMO or RRT. No additional dose of alteplase 0.3 mg/kg/2 h or 0.6 mg/kg/2 h (Part 1) or 0.6 mg/kg/2 h in Part 2 should be given under these circumstances.

Recommendation for temporary interruption of alteplase daily infusions:

- Interventions (e.g. insertion of lines, catheters, drains and tubes) during the treatment period with alteplase should be avoided, whenever possible. If absolutely necessary, they need to be performed in the alteplase infusion-free interval (i.e. in the 12-hours break between two infusions) and ideally not on the first day.
- Alteplase infusion can be resumed at least 6 hours after the intervention. This duration can be extended in case of complications during the intervention.
- If the fibrinogen level decreases to levels <150 mg/dL. The infusion should be restarted when fibrinogen level is 150 mg/dL or higher[c32479735-01; P13-13102]
- Interruptions for unavoidable interventions, drop in fibrinogen level or bleeding (beyond the 12 hours alteplase infusion-free interval per protocol) are allowed for up to a maximum of 72 hours. The time window of the subsequent visits and infusions may be extended accordingly. It is preferable to achieve normal haemostasis parameters before the next infusion of alteplase.

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:	**Initial i.v. infusion (Day 1 only):** **Part 1:** • Dosing regimen A: 0.3 mg/kg given over 2 hours • Dosing regimen B: 0.6 mg/kg given over 2 hours 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. <u>Dosing regimen A, Initial i.v. infusion (0.3 mg/kg to be infused over 2 h)</u> <u>Dosing regimen B, Initial i.v. infusion (0.6 mg/kg to be infused over 2 h)</u> **Part 2:** One dosing regimen is carried forward based on results from Part 1 and recommendation by the DMC for Part 2. <u>Dosing regimen B, Initial i.v. infusion (0.6 mg/kg to be infused over 2 h)</u>.

Table 4.1.1:1 Test product (cont)

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:	**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 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. Please note: On Day 1, the total infusion time is 2 hours plus 12 hours = 14 hours. Please note: If the 12-hour infusion has to be temporarily interrupted due to an urgent intervention, a drop of fibrinogen level < 150 mg/dL and / or bleeding, it can be restarted within the next 72 hours. If these interruptions extend beyond the protocol-recommended 12-hour alteplase-infusion free interval, the time-windows for subsequent long-term infusions move accordingly, and the last long-term infusion may be given later than day 5 in the study. 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. A new vial of alteplase should be taken every day.

Table 4.1.1:1 Test product (cont)

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:	**Dose of Long-Term i.v. infusion in Part 1:** Dosing regimen A: 0.02 mg/kg/hour given over 12 hours Dosing regimen B: 0.04 mg/kg /hour given over 12 hours **Dose of Long-Term i.v. infusion in Part 2:** One dosing regimen is carried forward based on results from Part 1 and recommendation by the DMC. Dosing regimen B: 0.04 mg/kg /hour given over 12 hours
	Optional additional i.v. infusion (allowed one time, during Days 2 to 5): **Part 1:** One optional additional **i.v. infusion:** **Dosing regimen A**: 0.3 mg/kg given over 2 hours **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) **Please note:** This optional single additional infusion of alteplase may only be given one time. This additional dose may be administered on either 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

Table 4.1.1:1 Test product (cont)

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
	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. For the optional additional **i.v. infusion** application, it is recommended that fibrinogen concentrations are ≥ 300mg/dL. **Part 2:** One dosing regimen is carried forward based on results from Part 1 and recommendation by the DMC. One optional additional **i.v. infusion:** **Dosing regimen B**: 0.6 mg/kg given over 2 hours This additional i.v. infusion can be given once during the treatment period in case of clinical worsening (as per investigator judgement)
	For Instructions for Preparation and Administration of Trial Medication, please see ISF. For dose calculation and infusion rates, please see Appendix 10.5
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 bellow 4.4 µM (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.

In Part 2 only dosing regimen B is applicable.

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). In Part 2, only dosing regimen B can be given as an additional i.v. infusion, as per investigator 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.
In Part 1 the randomisation will be stratified by the type of ventilation support (invasive mechanical / non-invasive) and in Part 2 by both type of ventilation support and by baseline D-dimer level (≥ULN to <5-fold ULN, versus ≥5-fold ULN), and access to the randomisation code will be controlled and documented.

Randomisation of IMV patients will be stopped when a maximum of 50 IMV patients have been randomized in Part 2.

The block sizes of the randomisation 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.

Part 1:

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.

Part 2:

Number of vials will be allocated per each patient depending on patient`s weight in treatment arm by IRT.

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 is carried forward based on results from Part 1 and recommendation by the DMC.

Initial i.v. infusion (Day 1):

- 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 (plus up to 72 hours in case of unexpected interruptions):

	Dosing regimen B
Target Dose	0.04 mg/kg/h

Optional additional i.v. infusion of 0.6 mg/kg infused over 2 hours (Dosing regimen B) is allowed once during the treatment period.

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 list 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 analysis 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:

Option A: Option A can only be applied in patients with an eGFR ≥ 30ml/min/1.73m^2.

Low-dose LMWH in prophylactic dosages given every day at the end of long-term i.v. alteplase infusion

Recommendations for LMWH dosages (these represent examples, and other LMWHs in prophylactic dosages are also allowed):

Dalteparin 5000 U, subcutaneously (SC) once daily (OD)
Enoxaparin 40 mg (4000 U), SC OD
Fondaparinux 2.5 mg, SC OD
Nadroparin 5700 U, SC OD (patients >70 kg)
Nadroparin 3800 U, SC OD (patients ≤70 kg)
Tinzaparin 4500 U, SC OD

In patients with moderate (creatinine clearance 30-50 ml/min/1.73m^2) and mild (creatinine clearance 50-80 ml/min/1.73m^2) renal impairment, careful clinical monitoring is advised.

or:

Option B: Option B can be chosen in all patients, irrrespective of their eGFR. Unfractionated heparin (UFH, e.g. 10 IU/ kg body weight/hour) concomitantly to long-term alteplase i.v. infusion, to be started 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].

UFH in a dosage of up to 5000 IU, SC BID (twice daily) can be given alternatively.

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

Heparin should not be given simultaneously with the initial 2h i.v. infusion of alteplase, or at time of an *optional* additional 2h i.v. infusion of alteplase, or when an intervention needs to be performed. The 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 interruption 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, in patients with mild to moderate renal impairment. The aPTT level should be monitored at least daily and 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).

Glucocorticoid treatment

Current or scheduled use of systemic (intravenous or oral) corticosteroid therapy should be included in the SOC regimen (e.g. 6 mg of dexamethasone orally) for up to 10 days or until hospital discharge. As patients in the alteplase treatment arm should also receive best possible SOC, this recommendation applies in the vs. non-invasive
alteplase arm as well.
Total daily dose equivalencies to dexamethasone 6 mg are provided in Appendix 10.6.

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 COVID-19 ARDS treatment.

For additional recommended SOC therapies please refer to Appendix 10.6.

Recommendations before the start of alteplase:

- Arterial lines and central venous catheters, tubes (e.g. for intubation and feeding) and drains (e.g. pleural and abdominal) should be placed at least 6 hours before starting the first treatment with alteplase.
- A delay of the alteplase treatment of approximately 6 hours after the insertion of the last line/catheter is recommended to allow for complete hemostasis (this time-period can be extended to up to 24 hours after complicated interventions)
- Before the start of the very first alteplase infusion, there should have been a time period of at least 10 hours since the last LMWH (S.C.) administration to ensure no significant overlap of the LMWH and alteplase.

Emergency procedures

Management of Bleeding during Alteplase Treatment:

- In case of bleeding, the alteplase infusion can be interrupted up to a maximum of 72 hours.
- A restart is possible, if complete haemostasis is achieved, and if lab results support a restart (fibrinogen ≥150mg/dL, haemoglobin is stable, anti-Xa 4 hours after s.c. administration is < 0.5, or aPTT ratio is < 1.5-fold of upper limit of normal).
- In case of interruptions of more than 12 hours, the time-windows for subsequent long-term infusions move accordingly, and the last long-term infusion may be given later than day 5 in the study. In total, up to 5x long-term infusions (of 12 hours each) can be given.

Management of serious bleeding events:

In case of serious bleeding, 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 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 (100 mg/dL) is desirable with cryoprecipitate infusion. Antifibrinolytic agents should also be considered.

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 should interrupt the alteplase infusion.

The alteplase infusion can be restarted, if fibrinogen recovers to ≥150 mg/dL during the following 72 hours.

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).
- LMWH/unfractionated heparins in therapeutic dosages
- 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 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 prior to randomisation.*

Except for the use of fibrinolytic drugs, 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 haemodynamically unstable 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). On Day 1 (randomisation day) an assessment just before randomisation shall be collected as baseline value. The last assessment per day should be recorded in the case report form.

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, PaO_2/FiO_2 ≥150 or SpO_2/FiO_2 ≥200	7
	Mechanical ventilation PaO_2/FiO_2<150 (SpO_2/FiO_2<200) or vasopressors	8
	Mechanical ventilation PaO_2/FiO_2 <150 and vasopressors, dialysis, or ECMO	9
Dead	Dead	10

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

5.1.2 Oxygen- free and Ventilator-free days, days in ICU

Oxygen-free and 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 PaO_2/FiO_2 ratio

The respiratory status of the enrolled patients is monitored by the PaO_2/FiO_2 ratio (or inferred PaO_2/FiO_2 ratio from SpO_2, see Appendix 10.2) regularly and all available data points will be collected until discharge, death, or Day 28, whatever occurs first. Fraction of Inspired Oxygen (FiO2) for common oxygen delivery systems are provided in Appendix 10.3.

During the first 6 days and during stay on ICU (whatever is longer) PaO_2/FiO_2 ratio should be measured three times per day in all patients, together with a recording of the patient`s position. The qualifying PaO_2/FiO_2 ratio should be the worst measurement in the screening period. The worst measurement on a given day should be documented in the case report form. 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 28. If patient is discharged from hospital prematurely, the last evaluation needs to be performed at the day of discharge from hospital. (Appendix 10.1)

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. More details on collection of safety laboratory parameters are summarized in Table 5.2.3:1
Patients do not have to be fasted for the blood sampling for the safety laboratory.

Table 5.2.3:1 Safety laboratory tests

Haematology
• Haematocrit • Haemoglobin • RBCs / Erythrocytes • White Blood Cells (WBC) / Leukocytes • Platelet Count / Thrombocytes
Clinical chemistry
• Albumin* • Alkaline phosphatase* • ALT (alanine transaminase, serum glutamic-pyruvic transaminase [SGPT]) • AST (aspartate transaminase, serum glutamic-oxaloacetic transaminase [SGOT]) • Bilirubin total* • Creatinine *(estimated GFR to be calculated according to local practice)* • 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
• 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 cells[1]

and/or

- Fatal bleed

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

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 (as 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. For patients on Alteplase treatment, these events should always be reported as serious adverse events, as described in Section 5.2.6.2. For patients on SOC, adverse events should be considered serious if they meet any of the seriousness criteria, outlined in Section 5.2.6.1.2.

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. Please refer to Section 5.2.6.2.4 for Exemptions to SAE reporting.

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.

The rules for Adverse Event Reporting exemptions still apply, please see Section 5.2.6.2.4.

5.2.6.2.2 Adverse event reporting to the sponsor and timelines

For the patients on Alteplase treatment arm, 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 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.

For patients not exposed to Alteplase treatment, but to SOC only: No expedited reporting via SAE form is required. All safety data (AEs and SAEs) should be included in the eCRF.

Consequently, the investigator is encouraged to report all adverse events for SOC group drugs in accordance to the local regulatory requirements for spontaneous AE reporting at the investigator's discretion by using their locally established routes and AE report forms.

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. A woman is considered of childbearing potential, i.e. fertile, following menarche and until becoming post-menopausal unless permanently sterile. Permanent sterilisation methods include hysterectomy, bilateral salpingectomy and bilateral oophorectomy. Tubal ligation is NOT a method of permanent sterilisation.
A postmenopausal state is defined as no menses for 12 months without an alternative medical cause.

5.2.6.2.4 Exemptions to SAE reporting

Disease progression of the underlying ARDS reflects the natural course of the disease and is exempted from reporting as an (S)AE. Progression of the subject's underlying ARDS will be recorded on the appropriate pages of the (e)CRF only and will not be reported as an SAE.
The specific preferred terms (PTs) that are exempt are:

- Acute respiratory distress syndrome
- Respiratory distress
- Acute respiratory failure
- Respiratory failure

Death due to disease progression is also to be recorded on the appropriate eCRF page and not on the SAE form. Disease progression and death due to disease progression will therefore not be entered in the safety database and hence not get expeditiously reported.
However, if there is evidence suggesting a causal relationship between the study drugs and the progression of the underlying ARDS, the event must be reported as SAE on the eCRF, as well as on the SAE form.
Exempted events will be monitored at appropriate intervals by the external DMC.

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. As PK samples collection included less than 10 patients per treatment arm in Part 1, collection of PK samples will continue in Part 2. It is intended to obtain PK samples from at least 17 patients receiving Alteplase 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.

[redacted]

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 clinical functional status.

All secondary [redacted] 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 qualifying PaO_2/FiO_2 ratio should be the worst measurement in the screening period.

For eligibility, patients should be hospitalized with the diagnosis of COVID-19 ARDS. SARS-CoV-2 laboratory confirmation (RT-PCR test) is a pre-requisite at screening. The RT-PCR test needs to be in timely association with the current hospital stay.

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

Randomisation of patients (Day 1, Visit 2a) shall happen not later than 24 hours after the confirmation of the inclusion and exclusion criteria. Study drug dispensing and administration shall start within 6 hours of randomisation. The time-point of obtaining D-Dimer levels can be as long as four calendar days before screening.

Treatment period duration is 5 days.

Interruptions for urgent reasons for a total of up to 72 hours are allowed and accordingly, the time window of the visits may be extended.

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 treatment period the worst measurement on a given day should be documented in the case report form. During the first 6 days and during the stay on ICU (whatever is longer), PaO_2/FiO_2 ratio 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 and oxygen 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 dosing regimen A”
- alteplase 0.6mg/kg over 2 hours, followed by daily long-term (12h) infusion of 0.04 mg/kg/h over 5 days, “alteplase dosing regimen B”
- 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. Accordingly, the following dosing regimen will be carried forward in Part 2: alteplase 0.6mg/kg over 2 hours, followed by daily long-term (12h) infusion of 0.04 mg/kg/h over 5 days, “alteplase dosing regimen B“
- standard of care

in a 2:1 ratio, stratified according to baseline ventilation status (invasive mechanical vs. non-invasive) and baseline D-Dimer level (≥ULN to <5 vs ≥5-fold ULN).

Throughout this whole Section, the NIV (non-invasive ventilation) patient cohort are those with a baseline WHO value of 6, and the IMV (invasive mechanical ventilation) patient cohort are those with a value 7, 8 or 9 at baseline.

7.1 NULL AND ALTERNATIVE HYPOTHESES

Throughout this Section, alteplase refers to the randomised treatment group, which is in addition to SOC. With regards to Part 1, reference will be made to alteplase dosing regimen A and alteplase dosing regimen B and in Part 2, reference will be made to alteplase.

The primary endpoint related to Part 1, secondary endpoints, [redacted] 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 key secondary objectives of the statistical analysis are to determine whether alteplase significantly prevents treatment failure (defined as WHO value ≥7) at Day 28, or all cause mortality at Day 28, in comparison to SOC alone.

For the primary analysis of the primary endpoint a Cox proportional hazards model will be used. In the NIV patient cohort, adjustment will be made for the number of days under NIV support, baseline D-Dimer level and age. For the IMV patient cohort, adjustment will be made for the baseline WHO value, baseline D-Dimer level and age.

For the primary analysis of the key secondary endpoints, the delta method and average marginal effect method will be used to calculate the risk differences, the 95% confidence intervals and p-values. Adjustment will be made for the same set of default covariates as in the primary analysis of the primary endpoint.

In line with the primary and key secondary objectives of the statistical analysis, a confirmatory hierarchical testing procedure will be applied for the following set of hypotheses, in the NIV patient cohort, in the following order:-

H0_1:
The null hypothesis to be tested for the primary endpoint in the NIV patient cohort is that the hazard ratio between alteplase and SOC alone equals 1 for all t>0. The alternative hypothesis is that the hazard ratio between alteplase and SOC alone is not equal to 1 for all t>0. Superiority will be declared if the hazard ratio between alteplase and SOC alone is significantly greater than 1 at the two-sided type I error level alpha=5%.

H0_2:
The null hypothesis to be tested for the key secondary endpoint ‘Treatment failure at Day 28’ in the NIV patient cohort is that the risk difference between alteplase and SOC alone equals 0. The alternative hypothesis is that the risk difference between alteplase and SOC alone is not equal to 0. Superiority will be declared if the risk difference between alteplase and SOC alone is significantly less than 0 at the two-sided type I error level alpha=5%.

H0_3:
The null hypothesis to be tested for the key secondary endpoint ‘All cause mortality at Day 28’ in the NIV patient cohort is that the risk difference between alteplase and SOC alone equals 0. The alternative hypothesis is that the risk difference between alteplase and SOC alone is not equal to 0. Superiority will be declared if the risk difference between alteplase and SOC alone is significantly less than 0 at the two-sided type I error level alpha=5%.

In the first step, the primary endpoint hypothesis (H0_1) will be tested at the 2-sided type I error level of alpha=5%. If the null hypothesis (H0_1) is rejected, the key secondary endpoint hypothesis (H0_2) based on the endpoint Treatment failure at Day 28 will then be tested in a second step, again at alpha=5%. If the null hypothesis (H0_2) is rejected, the remaining key secondary endpoint hypothesis (H0_3) based on the endpoint all cause mortality at Day 28 will then be tested in a third step, again at alpha=5%.

7.2 PLANNED ANALYSES

7.2.1 General considerations

With regards 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) but this PPS will only be determined for Part 2.

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

All analyses for all endpoints, both inferential and descriptive, including safety, will be performed on the NIV patient cohort, and the IMV patient cohort separately. This is in line with the statistical objectives of the study.

For selected endpoints, if homogeneity between the two patient cohorts is observed, combined treatment estimates will be determined using suitable meta-analysis techniques. These will be outlined in more detail in the Trial Statistical Analysis Plan.

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 dosing regimen A to SOC alone, and alteplase dosing regimen B 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 alteplase to SOC alone. From this model, the 95% confidence interval and corresponding Wald p-value for this HR will be produced. In the NIV cohort, adjustment will be made for the number of days under NIV support, baseline D-Dimer level and age. For the IMV cohort, adjustment will be made for the baseline WHO value, baseline D-Dimer level 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.

A sensitivity analysis of the primary endpoint (main estimand) will be carried out on the PPS, in line with the primary analysis.

Following the amendment to the inclusion criteria (CTP Version 4.0), whereby the original D-Dimer level changed from ≥3-ULN to ≥ULN, some additional analyses will be performed. Firstly, a sensitivity analysis will be added, similar to the primary analysis, just replacing the baseline D-Dimer status on the two levels with that on the three levels (≥ULN to <3, ≥3 to <5, ≥5-ULN). Secondly, a supplementary analysis will be added, similar to the primary analysis, but removing all patients with baseline D-dimer status ≥ULN to <3-ULN, which is according to the expanded inclusion criteria.

7.2.3 Secondary endpoint analyses

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

The key secondary endpoints are both binary in nature and will be analysed using frequency tables, risk differences, 95% confidence intervals and p-values. The delta method and average marginal effect (AME) method will be used. In the NIV cohort, adjustment will be made for the number of days under NIV support, baseline D-Dimer level and age. For the IMV cohort, adjustment will be made for the baseline WHO value, baseline D-Dimer level and age.

For the safety endpoint (MBE to Day 6) which is binary, the Chang and Zhang exact method will be used, without adjustment for covariates.

The continuous endpoints will be analysed descriptively and inferentially, albeit in a non-confirmatory manner. A restricted maximum likelihood (REML) based ANCOVA will be used. In the NIV cohort, adjustment will be made for the number of days under NIV support, baseline D-Dimer level and age. For the IMV cohort, adjustment will be made for the baseline WHO value, baseline D-Dimer level and age. The treatment difference with 95% confidence interval will be presented.
More detailed descriptions 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.

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 “dosing regimen A”
- alteplase 0.6 mg/kg over 2 hours followed by 0.04 mg/kg/h over12 hours “dosing regimen B”
- 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 alteplase and to standard of care, with an allocation ratio of 2:1, with stratification by the following factors:

- Type of ventilation (non-invasive or invasive mechanical)
- D-Dimer levels (≥ULN to <5-fold ULN, versus ≥5-fold ULN)

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.

Sample size considerations are limited to Part 2 and the NIV patient cohort.

Part 2 has a planned confirmatory analysis at the 2-sided type I error level of alpha=5% with a power of 85% to 90%, applicable to the first two steps within the pre-planned hierarchial testing procedure. The trial uses a randomisation allocation ratio of 2:1, which leads to approximately 140 NIV patients on the treatment arm and approximately 70 NIV patients on the SOC alone.

With regard to the primary analysis of the primary endpoint, H0_1 in the hierarchy testing procedure, in the NIV patient cohort, a hazard ratio of 2.0 is assumed, with event rates in the SOC alone group and alteplase group of approximately 30% and 50%. In the alteplase arm, it is expected to have a median time to event of 20 to 30 days.

With regard to the primary analysis of the key secondary endpoint “Treatment failure at Day 28 ”, H0_2 in the hierarchy testing procedure, in the NIV patient cohort, a risk difference of -20% is assumed, with event rates in the SOC alone group and alteplase group of approximately 40% and 20%.

With regard to the primary analysis of the key secondary endpoint “All cause mortality at Day 28”, H0_3 in the hierarchy testing procedure, in the NIV patient cohort, there would be a power of 85% under the assumptions of a risk difference of -16%. This would be with event rates in the SOC alone group and alteplase group of approximately 25% and 9%. These are very similar event rates as observed in Part 1.

The sample size determination or justification is in line with the null hypotheses and hierarchial testing approach as defined in Section 7.1, whereby it is planned to test for superiority of alteplase vs SOC alone in the NIV patient cohort. Whilst the assumptions are still rather approximate, the magnitude of these assumptions is similar to the available results

in other COVID-19 patients [R20-2001, R20-2246] and to the results observed in Part 1 of this study. In this emerging disease and during this pandemic, standard of care is evolving, and other factors are having an influence, for example, the introduction of vaccines.

Calculations were performed using the software R Version 3.6.1 and nQuery Version 4.0.

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 also is planned to be performed in at least 10 patients per dose group in Part 1 and in approximately 17 patients in Part 2. 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 **a**ttributable, **l**egible, **c**ontemporaneous, **o**riginal and **a**ccurate (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, IRB/EC review or regular inspections due to COVID-19 restrictions. 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. The rules for Adverse Event Reporting exemptions still apply, please see Section 5.2.6.2.4.

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 laboratorie`s 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 also review at specified intervals the exempted events to SAE reporting as specified in Section 5.2.6.2.4. 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.

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10. APPENDICES

10.1 SOFA SCORE AND GLASGOW COMA SCALE

The SOFA score [R20-1695] is calculated as follows:

	SOFA Score				
Variables	0	1	2	3	4
Respiratory Pao_2/Fio_2, mm Hg	>400	≤400	≤300	≤200†	≤100†
Coagulation Platelets $\times 10^3/\mu L$‡	>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 Fio_2, 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.

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 TABLE TO DERIVE PaO_2/FiO_2 RATIO FROM SpO_2

The PaO_2/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

10.2 TABLE TO DERIVE PAO2/FIO2 RATIO FROM SPO2 (cont)

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%.	

10.3 FIO_2 RANGES FOR COMMON OXYGEN DELIVERY DEVICES

Fraction of Inspired Oxygen (FiO_2) for common oxygen delivery systems are given in the tables below [R22-0456]:

Device	Flow (L/min)	F_{IO_2}
Nasal cannula	1–6	0.24–0.40
Simple mask	5–10	0.35–0.50
Non-rebreather mask	10–15	0.60–0.80
OxyMask	1.5–15	0.25–0.80

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). 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 the nature of this trial and the risks associated with Covid-19, site monitoring visits as physical on-site interaction may not always be possible. The focus will be on centralized oversight/monitoring. If not restricted by local Covid-19 regulations, we recommend combination of remote and on-site visits, mainly for initiation and close-out visits. Regular interim monitoring visits will occur mainly remotely unless the trial team requires a visit to be done on-site due to an identified risk where the required mitigation may need to be performed on-site.
Trial monitoring intervals may be adjusted depending on enrolment rates, data quality, recruitment and conduct phases, timelines for CRF completion and/or site quality.

10.5 ALTEPLASE DOSING TABLE

Dosing regimen A (lower dose) dosing table (only applicable in Part 1 of the trial)

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)	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

10.5 ALTEPLASE DOSING TABLE (cont)

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)	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).

10.5 ALTEPLASE DOSING TABLE (cont)

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)	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

10.5 ALTEPLASE DOSING TABLE (cont)

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)	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

*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).

10.6 TREATMENT RECOMMENDATIONS FOR COVID-19 ARDS (WITH A FOCUS ON NIV PATIENTS)

Standard of Care should be harmonized as much as possible in each center and across centers. Changes in the Standard of Care during the study period are acceptable, if new evidence arises and/or new recommendations are published during this period.

Recommendations given below are based on international treatment recommendation as of September 2021. The Table below is intended to give the investigator an updated status of therapeutics used in COVID 19 that are recommended for local implementation.

Rating of Recommendations: A = Strong; B = Moderate; C = Optional.
Rating of Evidence: I = One or more randomized trials without major limitations; IIa = Other randomized trials or subgroup analyses of randomized trials; IIb = Nonrandomized trials or observational cohort studies; III = Expert opinion

Table 10.6:1 Treatment Recommendations for COVID-19 ARDS (with a focus on NIV Patients)

SYSTEMIC CORTICOSTEROIDS	
RECOMMEN-DATION	• The WHO-Guideline recommends systemic corticosteroids rather than no systemic corticosteroids for the treatment of patients with severe and critical COVID-19 (strong recommendation, based on moderate certainty evidence). • The NIH- Guideline recommends one of the following options for patients who require delivery of oxygen through a high-flow device or noninvasive ventilation but not invasive mechanical ventilation or extracorporeal membrane oxygenation o Dexamethasone (AI); or o Dexamethasone plus remdesivir (BIII). The combination of remdesivir plus dexamethasone has not been rigorously studied in clinical trials; therefore, the safety and efficacy of this combination are unknown.

Table 10.6:1 Treatment Recommendations for COVID-19 ARDS (with a focus on NIV Patients) (cont)

SYSTEMIC CORTICOSTEROIDS	
ADDITIONAL CONSIDERATIONS	• Immunosuppressive therapy (e.g., dexamethasone with or without baricitinib or tocilizumab) may increase the risk of opportunistic infections or reactivation of latent infections; however, randomized trials to date have not demonstrated an increase in the frequency of infections. • Cases of severe and disseminated strongyloidiasis have been reported in patients with COVID-19 during treatment with tocilizumab and corticosteroids. Many clinicians would initiate empiric treatment for strongyloidiasis (e.g., with ivermectin) with or without serologic testing in patients from areas where Strongyloides is endemic (i.e., tropical, subtropical, or warm temperate areas). For contraindications, special warnings and precautions for use please refere to the label and further references.
DOSING	Dexamethasone 6 mg IV or PO once daily for up to 10 days or until hospital discharge. If dexamethasone is not available, alternative glucocorticoids (e.g., prednisone, methylprednisolone, hydrocortisone) can be used. For these drugs, the total daily dose equivalencies to dexamethasone 6 mg (oral or intravenous) are: • Prednisone 40 mg • Methylprednisolone 32 mg • Hydrocortisone 160 mg
REFERENCES	R20-4049, R21-2944, P22-01013, R22-0477

Table 10.6:1 Treatment Recommendations for COVID-19 ARDS (with a focus on NIV Patients) (cont)

IL-6 INHIBITORS (E.G. TOCILIZUMAB OR SARILUMAB)	
RECOMMENDATION	• The WHO-Guideline recommends treatment with IL-6 receptor blockers (tocilizumab or sarilumab) for patients with severe or critical COVID-19 infection. The WHO recommends patients meeting severity criteria should receive both corticosteroids and IL-6 receptor blockers. • The NIH- Guideline recommends one of the following options for patients who require delivery of oxygen through a high-flow device or noninvasive ventilation but not invasive mechanical ventilation or extracorporeal membrane oxygenation o Dexamethasone (AI); or o Dexamethasone plus remdesivir (BIII). • For patients who have rapidly increasing oxygen needs and have increased markers of inflammation, add either baricitinib (BIIa) or tocilizumab (BIIa) (drugs are listed alphabetically) to 1 of the 2 options above. • The Panel recommends against the use of baricitinib in combination with tocilizumab for the treatment of COVID-19, except in a clinical trial (AIII). Because both baricitinib and tocilizumab are potent immunosuppressants, there is the potential for an additive risk of infection.
ADDITIONAL CONSIDERATIONS	• Immunosuppressive therapy (e.g., dexamethasone with or without baricitinib or tocilizumab) may increase the risk of opportunistic infections or reactivation of latent infections; however, randomized trials to date have not demonstrated an increase in the frequency of infections. • Cases of severe and disseminated strongyloidiasis have been reported in patients with COVID-19 during treatment with tocilizumab and corticosteroids. Many clinicians would initiate empiric treatment for strongyloidiasis (e.g., with ivermectin) with or without serologic testing in patients from areas where Strongyloides is endemic (i.e., tropical, subtropical, or warm temperate areas).

Table 10.6:1 Treatment Recommendations for COVID-19 ARDS (with a focus on NIV Patients) (cont)

IL-6 INHIBITORS (E.G. TOCILIZUMAB OR SARILUMAB)	
ADDITIONAL CONSIDERATIONS	• Baricitinib or tocilizumab should only be given in combination with dexamethasone or another corticosteroid. Some clinicians may assess a patient's clinical response to dexamethasone before deciding whether adding baricitinib or tocilizumab as a second immunomodulatory drug is necessary. • Studies that directly compare baricitinib to tocilizumab as treatments for COVID-19 are not available. Therefore, the Panel has insufficient evidence to recommend one drug over the other. Treatment decisions should be made based on local guidance, drug availability, and patient comorbidities. • Even though the REMAP-CAP trial supports that sarilumab and tocilizumab have similar efficacy in the treatment of hospitalized patients with COVID-19, the Panel recommends sarilumab only when tocilizumab is not available or is not feasible to use (BIIa) For contraindications, special warnings and precautions for use please refere to the label and further references.
DOSING	Tocilizumab is dosed at 8 mg per kilogram of actual body weight, up to a maximum of 800 mg. Sarilumab is most commonly dosed at 400 mg.
REFERENCES	R21-2936, R21-2943, P22-01013, R22-0477

Table 10.6:1 Treatment Recommendations for COVID-19 ARDS (with a focus on NIV Patients) (cont)

BARICITINIB (JAK INHIBITOR)	
RECOMMENDATION	Please see recommendations for IL6-Inhibitors above.
ADDITIONAL CONSIDERATIONS	Please see additional considerations for IL6-Inhibitors above. • The Panel recommends against the use of the combination of baricitinib and tocilizumab for the treatment of COVID-19 except in a clinical trial (AIII), because there is insufficient evidence for the use of this combination. Given that both baricitinib and tocilizumab are potent immunosuppressants, there is the potential for an additive risk of infection. • If baricitinib and IV tocilizumab are not available or not feasible to use, tofacitinib can be used instead of baricitinib (BIIa) and IV sarilumab can be used instead of IV tocilizumab (BIIa). For contraindications, special warnings and precautions for use please refere to the label and further references.
DOSING	• Baricitinib dose is dependent on eGFR; duration of therapy is up to 14 days or until hospital discharge. eGFR ≥60 mL/min/1.73 m^2: Baricitinib 4 mg PO once daily eGFR 30 to <60 mL/min/1.73 m^2: Baricitinib 2 mg PO once daily eGFR 15 to <30 mL/min/1.73 m^2: Baricitinib 1 mg PO once daily eGFR <15 mL/min/1.73 m^2: Baricitinib is not recommended.
REFERENCES	R21-3171, R21-3178, P22-01013, R22-0477
PROPHYLACTIC ANTICOAGULATION FOR PREVENTION OF DVT/VTE	
RECOMMENDATION	• The results of three international randomized controlled trials (REMAP-CAP, ACTIV-4a, and ATTACC) demonstrated that therapeutic-dose of anticoagulation with heparin in patients hospitalized with COVID-19 did not prevent progression of disease or death in patients with critical COVID-19 who are in the ICU, and that this dosing regimen might be associated with increased rates of significant bleeding [R21-2851].
REFERENCES	R21-2851

Table 10.6:1 Treatment Recommendations for COVID-19 ARDS (with a focus on NIV Patients) (cont)

OXYGENATION, VENTILATION AND AWAKE PRONE POSITIONING	
RECOMMENDATION	The COVID-19 Treatment Guidelines Panel (NIH Panel) and the Surviving Sepsis Campaign (SSC) recommend: • For adults with COVID-19 and acute hypoxemic respiratory failure despite conventional oxygen therapy, the Panel recommends HFNC oxygen over NIV (BIIa). • For adults with COVID-19 and acute hypoxemic respiratory failure who do not have an indication • for endotracheal intubation and for whom HFNC oxygen is not available, the Panel recommends performing a closely monitored trial of NIV (BIIa). • For patients with persistent hypoxemia who require HFNC oxygen and for whom endotracheal intubation is not indicated, the Panel recommends a trial of awake prone positioning (BIIa). The Panel recommends against using awake prone positioning as a rescue therapy for refractory hypoxemia to avoid intubation in patients who otherwise meet the indications for intubation and mechanical ventilation (AIII). If intubation becomes necessary, the procedure should be performed by an experienced practitioner in a controlled setting due to the enhanced risk of exposing health care practitioners to SARS-CoV-2 during intubation (AIII).
REFERENCES	R21-2938, R21-3192, P22-01013, R21-3191, R22-0473

Table 10.6:1 Treatment Recommendations for COVID-19 ARDS (with a focus on NIV Patients) (cont)

HEMODYNAMICS	
RECOMMENDATION	The COVID-19 Treatment Guidelines Panel (NIH Panel) and the Surviving Sepsis Campaign (SSC) recommend: • For adults with COVID-19 and shock, the Panel recommends using dynamic parameters, skin temperature, capillary refilling time, and/or lactate levels over static parameters to assess fluid responsiveness (BIIa). • For the acute resuscitation of adults with COVID-19 and shock, the Panel recommends using buffered/balanced crystalloids over unbalanced crystalloids (BIIa). • For the acute resuscitation of adults with COVID-19 and shock, the Panel recommends against the initial use of albumin for resuscitation (BI). • For adults with COVID-19 and shock, the Panel recommends norepinephrine as the first-choice vasopressor (AI). • For adults with COVID-19 and shock, the Panel recommends titrating vasoactive agents to target a mean arterial pressure (MAP) of 60 to 65 mm Hg over higher MAP targets (BI). • The Panel recommends against using hydroxyethyl starches for intravascular volume replacement in patients with sepsis or septic shock (AI). • When norepinephrine is available, the Panel recommends against using dopamine for patients with COVID-19 and shock (AI). • As a second line vasopressor, the Panel recommends adding either vasopressin (up to 0.03 units/min) (BIIa) or epinephrine (BIIb) to norepinephrine to raise MAP to target or adding vasopressin (up to 0.03 units/min) (BIIa) to decrease norepinephrine dosage. • The Panel recommends against using low-dose dopamine for renal protection (AI). • The Panel recommends using dobutamine in patients who show evidence of cardiac dysfunction and persistent hypoperfusion despite adequate fluid loading and the use of vasopressor agents (BIII). • The Panel recommends that all patients who require vasopressors have an arterial catheter placed as soon as practical, if resources are available (BIII).

Table 10.6:1 Treatment Recommendations for COVID-19 ARDS (with a focus on NIV Patients) (cont)

	• For adults with refractory septic shock who have completed a course of corticosteroids to treat their COVID-19, the Panel recommends using low-dose corticosteroid therapy ("shock-reversal") over no corticosteroid therapy (BIIa).
REFERENCES	P22-01013, R21-3191, R22-0473
EMPIRIC BROAD-SPECTRUM ANTIMICROBIAL THERAPY	
RECOMMENDATION	NIH guideline recommendations: • In patients with COVID-19 and severe or critical illness, there is insufficient evidence for the Panel to recommend either for or against empiric broad-spectrum antimicrobial therapy in the absence of another indication. • If antimicrobials are initiated, the Panel recommends that their use should be reassessed daily to minimize the adverse consequences of unnecessary antimicrobial therapy (AIII).
REFERENCES	P22-01013
ACUTE KIDNEY INJURY AND RENAL REPLACEMENT THERAPY	
RECOMMENDATION	NIH guideline recommendations: • For critically ill patients with COVID-19 who have acute kidney injury and who develop indications for renal replacement therapy, the Panel recommends continuous renal replacement therapy (CRRT), if available (BIII). • If CRRT is not available or not possible due to limited resources, the Panel recommends prolonged intermittent renal replacement therapy rather than intermittent hemodialysis (BIII).
REFERENCES	P22-01013
EXTRACORPOREAL MEMBRANE OXYGENATION	
RECOMMENDATION	NIH guideline recommendations: • There is insufficient evidence for the Panel to recommend either for or against the use of extracorporeal membrane oxygenation for patients with COVID-19 and refractory hypoxemia.
REFERENCES	P22-01013

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		The **TRISTARDS** trial - **Th**rombolys**IS** **T**herapy for **ARDS** A Phase IIb/III operationally seamless, open-label, randomised, sequential, parallel-group adaptive study to evaluate the efficacy and safety of daily intravenous alteplase treatment given up to 5 days on top of standard of care (SOC) compared with SOC alone, in patients with acute respiratory distress syndrome (ARDS) triggered by COVID-19.
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		Vital signs and laboratory tests added at Day 6 IRT call and drug dispensation at Day 2,3 and 4 *changed to:* removed
Rationale for change		Mistakenly entered for IRT call; vital signs and laboratory tests added at Day 6 because these are required for SOFA [redacted] score

		calculation.
Section to be changed		Flowchart , Footnote 6
Description of change 2		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. *changed to:* On Day 6 only lab samples required for [redacted], SOFA score and PaO_2/FiO_2 shall be collected.
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		[redacted] and SOFA will be assessed until Day 6 *changed to:* 1. [redacted] nd 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		ARDS with PaO_2*/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 and CT scan (not fully explained by effusions, lobar/lung collapse, or nodules) - *changed to:* - 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 *Changed to:* 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 *Changed to:* 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 *Changed to:* Covid-19
Rationale for change		Typo correction

11.2 GLOBAL AMENDMENT 2

Date of amendment		01 Oct 2021
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		The **TRISTARDS** trial - **Th**rombolys**IS T**herapy for **ARDS** A Phase IIb/III operationally seamless, open-label, randomised, sequential, parallel-group adaptive study to evaluate the efficacy and safety of daily intravenous alteplase treatment given up to 5 days on top of standard of care (SOC) compared with SOC alone, in patients with acute respiratory distress syndrome (ARDS) triggered by COVID-19.

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		Protocol Synopsis
Description of change 1		Added and updated: o Key-secondary endpoints o Secondary endpoints o Number of patients in Part 2 updated / planned number of patients on NIV/ IMV updated o Randomisation additionally stratified by D-Dimer status o Alteplase labelled to alteplase dosing regimen B o Updated Inclusion/Exclusion criteria

		○ Treatment duration update Statistical methods : Part 2 will consist of two cohorts, the NIV patient cohort and the IMV patient cohort, and these two cohorts will be analysed separately. Only the NIV patient cohort will be part of the main statistical objectives of the study, and therefore part of the confirmatory hierarchical testing procedure. In Part 2, a confirmatory hierarchical testing procedure in the NIV patient cohort will be applied for the primary endpoint and the two key secondary endpoints. In a first step, the primary endpoint hypothesis in NIV patients will be tested. If the null hypothesis is rejected, the key secondary endpoint of Treatment failure at Day 28 will then be tested in the NIV patient cohort as a second step. If this null hypothesis is rejected, the key secondary endpoint All cause mortality at Day 28 will then be tested in the NIV patient cohort as a third step. In the NIV cohort, adjustment will be made for the number of days under NIV support, baseline D-Dimer level and age. For the IMV cohort, adjustment will be made for the baseline WHO value, baseline D-Dimer level and age. For each of the two key secondary endpoints, treatment failure at Day 28 and all cause mortality at Day 28, the delta method and average marginal effect method will be used to calculate the risk difference, 95% confidence intervals and p-values. Adjustment will be made for the same set of covariates as in the primary analysis of the primary endpoint.
Rationale for change		Reflecting results of part 1 and DMC recommendations
Section to be changed		Flowchart, Footnote 3
Description of change 2		The time-point of obtaining D-Dimer levels can be as long as two calendar days before randomisation added
Rationale for change		Increase flexibility for D-dimer level assessment to confirm eligibility of patients

Section to be changed		Flowchart, Footnote 4
Description of change 3		Updated dose of Alteplase used in Part 2, update on treatment duration as well as duration of treatment interruptions
Rationale for change		Update based on results of Phase III for dose regimen and additional explanation for sites in case treatment interruption for safety reasons is needed
Section to be changed		Flowchart, Footnote 8
Description of change 4		Updated wording for anti-Xa measurements requirements and timing, frequency and requirements for aPTT measurement.
Rationale for change		Concomitant medication with LMWH is limited to prophylactic dosages only. Based on labelling information for LMWHs, monitoring of anti-Xa levels is not required in general. Monitoring of anti-Xa levels is adviced for patients with mild to moderate renal impairment according to the prescribing information of LMWHs.
Section to be changed		Flowchart, Footnote 9
Description of change 5		Added instructions for PaO_2/FiO_2 measurement on during screening and treatment period: o The qualifying PaO_2/FiO_2 ratio should be the worst measurement in the screening period. During the treatment period the worst measurement on a given day should be documented in the case report form. During the first 6 days and during the stay on ICU (whatever is longer), PaO_2/FiO_2 ratio should be measured at least three times daily in all patients.
Rationale for change		To ensure consistency of data collection across the sites
Section to be changed		Flowchart, Footnote 10
Description of change 6		[redacted]; SOFA score assessment only at baseline, Day 6 and Day 28. Additional instructions on SOFA score collection added
Rationale for change		Streamline operational feasibility. [redacted]

		[redacted] Assessment of SOFA was prolonged until Day 28.
Section to be changed		Flowchart, Footnote 11
Description of change 7		Instructions on timing of baseline and the last measurement of WHO score on a given day added
Rationale for change		To ensure consistency of WHO score assessment and recording across the sites
Section to be changed		Flowchart, Footnote 12
Description of change 8		Added: A woman is considered of childbearing potential, i.e. fertile, following menarche and until becoming post-menopausal unless permanently sterile. Permanent sterilisation methods include hysterectomy, bilateral salpingectomy and bilateral oophorectomy. Tubal ligation is NOT a method of permanent sterilisation. A postmenopausal state is defined as no menses for 12 months without an alternative medical cause.
Rationale for change		More detailed definition of 'women of childbearing potential' requested by regulatory authorities.
Section to be changed		Flowchart, Footnote 13
Description of change 9		The RT-PCR test needs to be in timely association with the current hospital stay. For eligibility, patients should be hospitalized with the diagnosis of COVID-19 ARDS. Removed COVID-19 RT-PCR test on Day 28
Rationale for change		More precise definition of RT-PCR test timing before screening of potential patients. Removal of COVID-19 test to reduce complexity and operational burden for sites
Section to be changed		Flowchart, Footnote 14
Description of change 10		Added: The rules for Adverse Event Reporting exemptions still apply, please see Section 5.2.6.2.4.
Rationale for change		Exemptions for AE reporting in SOC group as no investigational medicinal product is administered
Section to be changed		Section 1.1

Description of change 11		Added: o Discussion on evolving treatment options since Covid-19 outbreak o Critically ill COVID-19 patients with hypercoagulable state, presenting with a significant increase in D-dimer levels, might benefit from a thrombolytic therapy on top of anticoagulation with unfractionated or low-molecular weight heparins o Discussion of Part 1 / Phase III. The study results of Part 1 support the continuation of the study into Phase III (Part 2), implementing a comparison of the alteplase 0.6/0.04 mg/kg dose versus SOC alone.
Rationale for change		Positioning of the use of Alteplase treatment in the context of evolving treatment options in COVID-19, also including the results of Part 1 of the trial
Section to be changed		Section 1.2
Description of change 12		**Added:** Alteplase is currently indicated for the thrombolytic treatment of acute MI, acute massive PE **with haemodynamic instability and** acute ischemic stroke. The results of ECASS III show a positive net-clinical benefit for alteplase in patients with **acute ischemic** stroke.
Rationale for change		More precise wording
Section to be changed		Section 1.3
Description of change 13		The results of Part 1 of the current study added: **were intended** to provide proof of concept and establish the dose for Part 2, the confirmatory part of the study. The study results of Phase III of the current trial (Part 1) provided proof of concept and support the continuation of the study into Phase III (Part 2), the confirmatory part of the study, implementing a comparison of the alteplase 0.6/0.04 mg/kg dose versus SOC alone. Pharmacokinetic measurements **updated**: in both parts of the trial
Rationale for change		Provided reasoning of continuation of study from

		proof of concept to confirmatory part of the trial. Continuation of pharmacokinetic measurements in Part 2 due to too sparse samples collected in Part 1 of the study.
Section to be changed		Section 1.4.1 Benefits
Description of change 14		**Added:** o The results of Phase III of the current trial showed that patients receiving a dose of 0.6 mg/kg over 2 hours inmediately followed by a constant rate infusion of alteplase 0.04 mg/kg/h over 12 hours (up to 5 days) in addition to SOC was associated with faster clinical improvement or hospital discharge, compared with patients receiving alteplase 0.3/0.02 mg/kg on top of SOC, or SOC alone . The study results of Phase III (Part 1), support the continuation of the study into Phase III (Part 2), the confimartory part of the study. o Alteplase can be given in combination with UFH or LMWH**.** o During the alteplase treatment period of up to a maximum of 5 days (plus a possible extension in case of unavoidable interruptions of up to 72 hours) the concomitant use of LMWH in Part 2 is limited to prophylactic doses and the use of UFH is limited to target levels of aPTT between 1.0 to 1.5 fold ULN according to local laboratory. o Treating patients with a moderate initial dose of 0.6 mg/kg over 2 hours (for example, this corresponds to 42 mg in a patient with 70 kg of body weight) combined with a constant rate infusion (CRI) of low dose alteplase of 0.04 mg/kg/h over 12 hours is considered to reduce the risk of alteplase-related bleeding events in comparison to application of high alteplase doses (up to 100 mg) used in other indications (e.g. massive pulmonary embolism with hemodynamic instability, acute ischemic stroke, acute myocardial infarction). o These patients **replaced** by COVID-19

		ARDS
Rationale for change		Summarizing results of Part 1 supporting benefit of treatment with Alteplase 0.6 mg/kg over 2 hours inmediately followed by a constant rate infusion of alteplase 0.04 mg/kg/h over 12 hours (up to 5 days) in addition to SOC versus SOC alone.
Section to be changed		Section 1.4.2
Description of change 15		**Added**: 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 60 minutes were symptomatic intracerebral haemorrhage (6%) and other major haemorrhage (2%). Minor haemorrhage (e.g. bleeding at site of venepuncture) accounting for 20-25%. Similarly in patients with acute MI where alteplase doses (up to 100 mg) given over 90 min were applied, the risk of intracranial haemorrhage is <1%.In the Phase III part of the current trial, no new safety signals were identified at the chosen alteplase dose levels that are in general considerably lower than in approved indications (e.g. stroke, MI, PE). Major bleeding events until Day 6 were overall infrequent (1 patient in the 0.3/0.02 mg/kg dosing group and 3 patients in the 0.6/0.04 mg/kg dosing group), and a trend towards a dose-dependent increase was mainly observed for non-major bleeding events. Mitigation strategies to prevent bleeding events in Phase III (Part 2) of the current study are implemented in Section 3.3.4.1.
Rationale for change		No new safety signals observed in Part 1 of the trial, an increased risk of bleeding was observed in Part 1 and accordingly mitigation strategies are now adopted to prevent bleeding events that may occur in Part 2.
Section to be changed		Table 1.4.2:1
Description of change 16		Added possible or known risks: o Major bleeding including intracerebral

		haemorrhage or **bleeding from a critical organ** Mitigation strategy added:
Rationale for change		Safety information from CCDS added
Section to be changed		Section 1.4.3
Description of change 17		**Added:** o The safety results from Phase III (part 1) of the current trial were generally comparable across the treatment groups, except for bleeding events, which are a known side effect of alteplase. In Phase III, mainly non-major bleeding events were observed, and some of them were related to interventions. Additional risk minimization strategies to avoid intervention-related bleeding events have accordingly be implemented for Part 2 of the study. No new safety signals were identified. Major bleeding events in Part 1were overall infrequent. o If the level of fibrinogen falls alteplase has to be temporarily interrupted . o The study results of Phase III (Part 1), support the continuation of the study into Phase III (Part 2), the confimartory part of the study. The alteplase 0.6/0.04 mg/kg dose in addition to SOC was associated with faster clinical improvement or hospital discharge, compared with the alteplase 0.3/0.02 mg/kg + SOC, or SOC alone treatments. Patients receiving NIV support appeared to derive greater benefits than those receiving IMV support. The safety results were generally comparable across the treatment groups. No new safety signals were identified. Major bleeding events were overall infrequent, and a trend towards a dose-dependent increase was mainly observed for non-major bleeding events. o Precautionary recommendations for temporary interruption of alteplase infusion (up to 72 hours in total for urgent reasons, bleeding or drop in fibrinogen), a limitation of concomitant administration of heparins to prophylactic doses and recommendations to

		avoid intervention-related bleeding eventsare implemented in Part 2 to mitigate the risk of bleeding. o Taken together, the overall benefit-risk ratio of alteplase at the studied dosing regimen is considered favourable.
Rationale for change		Discussion of benefits and risks and summary of study results to support Part 2 study design. Justification of the dose of alteplase that was chosen for Part 2 of this trial.
Section to be changed		Section 2.1.3
Description of change 18		**Added:** In Part 2, the following key secondary endpoints will be evaluated: • Treatment failure defined as all cause mortality or mechanical ventilation at Day 28 • All cause mortality at Day 28 In Part 2, the following secondary endpoints will be evaluated: • Number of oxygen-free days up to Day 28 • Length of hospital stay up to Day 28 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. • ~~P~~aO_2/FiO_2 ratio (or inferred PaO_2/FiO_2 ratio from SpO_2) change from baseline up to Day 6
Rationale for change		Reflecting interim analysis and results of Part 1. Implementation of harder endpoints introduced as key secondary endpoints and implementation of a hierarchical testing approach to increase strength of the Phase III results.
Section to be changed		[redacted]
Description of change 19		

		[redacted]
Rationale for change		
Section to be changed		Section 3.1
Description of change 20		Added characteristics of patient population in Part 2 and treatment regimen to be used . Stratification used in Part 2 updated and recruitment cap for patients on IMV and NIV added .
Rationale for change		Reflecting results of Part 1 and DMC recommendation
Section to be changed		Figure 3.1.1
Description of change 21		A schematic overview of trial design adapted for part 2 – selected dose for alteplase added and planned number of patients adapted
Rationale for change		Reflecting results of Part 1 and DMC recommendation, reflecting sample size in Part 2
Section to be changed		Section 3.2
Description of change 22		Added reasoning of trial Protocol modifications: As Part 1 results indicated the need for some design modifications in Part 2 beyond those pre-specified in the protocol, these required modifications have been implemented via current global CTP amendment. DMC recommendation for Alteplase dosing regimen B added
Rationale for change		Reflecting results of Part 1 and DMC recommendation
Section to be changed		Section 3.3
Description of change 23		Updated number of participating sites in part 2 to 110-130. Added information on randomisation cap for IMV patients: In Part 2 of the trial: In part 2 of the trial, a randomisation cap will be applied for patients on mechanical ventilation at baseline: when 50 patients on mechanical ventilation have been randomized. Recruitment of further patients on mechanical ventilation will be stopped

		via IRT. Randomisation for the whole trial will be stopped when approximately 210 NIV patients have been randomized in Part 2, regardless of how many IMV patients have been randomised so far.
Rationale for change		Feasibility results show need to increase number of participating sites from regions with higher predicted incidence rate of Covid-19 infection. Randomisation cap was set-up reflecting results of Part 1 and recommendation of expert board to focus the main analyses on NIV patients.
Section to be changed		Section 3.3.2
Section to be changed		Inclusion criteria
Description of change 24		Removed: *-Eligibility criteria for Parts 1 and 2 are the identical* Inclusion criteria #1 updated: above > 18 (or above legal age)
Rationale for change		UK is no longer participating in the trial, but legal age above 16 may be applicable for other participating countries
Section to be changed		Exclusion criteria 2 and 7
Description of change 25		Added following exclusion criteria: o Indication for therapeutic dosages of anticoagulants at trial entry o Planned interventions during the first 5 days after randomisation, such as surgery, insertion of central catheter or arterial line, drains, etc.
Rationale for change		To avoid potential safety risk to patients
Section to be changed		Exclusion criterion #17
Description of change 26		Severe hepatic dysfunction - added i.e. Child-Pugh B and C
Rationale for change		To be in line with CCDS
Section to be changed		Exclusion criterion #23
Description of change 27		Malignancy (Stage IV) added: “with increased

		bleeding risk”
Rationale for change		To be in line with CCDS
Section to be changed		Section 3.3.4.1
Description of change 28		Need for ECMO or RRT. No additional dose of alteplase 0.3 mg/kg/2 h or 0.6 mg/kg/2 h (Part 1) or 0.6 mg/kg/2 h in Part 2 should be given under these circumstances.
Rationale for change		Clarification how to proceed with treatment in case patient requires ECMO or RRT: no additional 2h infusion of alteplase should be given
Section to be changed		Section 3.3.4.1
Description of change 29		Recommendations for temporary interruption of alteplase daily infusions updated.
Rationale for change		Provide sites with more detailed instructions for temporary interruption of treatment in case of interventions needed to be performed during treatment with alteplase
Section to be changed		Section 4.1.1 / Tab. 4.1.1:1
Description of change 30		Part 2 added: o Dosing regimen B, Initial i.v. infusion (0.6 mg/kg to be infused over 2 h). o Instructions for interruption of infusion adapted o Instructions for additional infusion added: i.v. infusion can be given once during the treatment period in case of clinical worsening (as per investigator judgement)
Rationale for change		Selected dosing regimen based on results of Part 1 of the trial and operational instructions in case of urgent interventions adapted to allow more flexibility and prevent treatment discontinuation.
Section to be changed		Section 4.1.2
Description of change 31		Dosing regimen B added as well as instructions that in Part 2, only dosing regimen B can be given as an additional i.v. infusion, as per investigator judgement
Rationale for change		Added selected dose for Part 2; clarification on dose

		to be used in Part 2
Section to be changed		Section 4.1.3
Description of change 32		Randomisation cap for patients on IMV has been added.
Rationale for change		Reflecting trial results from Part 1 and recommendation of expert board
Section to be changed 33		Section 4.1.4
Description of change		Added wording for Part 2: Number of vials will be allocated per each patient depending on patient`s weight in treatment arm by IRT.
Rationale for change		Inform sites on IMP allocation planned in Part 2 based on results of Part 1
Section to be changed		Section 4.2.1
Description of change 34		**Alteplase arm, Day 1 to 5 (treatment period):** The investigator may choose between Option A or B: **Added wording:** **<u>Option A</u>**: Option A can only be applied in patients with an eGFR ≥ 30ml/min/1.73m^2. Low-dose LMWH: added: **in prophylactic dosages** given every day at the end of long-term i.v. alteplase infusion Recommendations for LMWH dosages (these represent examples, and other LMWHs in prophylactic dosages are also allowed) provided **Recommendation added:** Before the start of the very first alteplase infusion, there should have been a time period of at least 10 hours since the last LMWH (S.C.) administration to ensure no significant overlap of the LMWH and alteplase. In patients with moderate (creatinine clearance 30-50 ml/min/1.73m^2) and mild (creatinine clearance 50-80 ml/min/1.73m^2) renal impairment, careful clinical monitoring is advised.

		Option B: Option B can be chosen in all patients, irrrespective of their eGFR. **Alteplase arm, post treatment period (Day 5 and onwards), and SOC arm:** Recommendation for anti-Xa monitoring: The anti Xa level should be monitored regularly, in patients with mild to moderate renal impairment. aPTT should be measured at least daily or more often
Rationale for change		Implementation of risk mitigation strategies to prevent bleeding events by the limitation to prophylactic dosages of LMWH only. Advice to monitor dosage of LMWH by anti-Xa levels in patients with mild to moderate renal impairment according to LMWH labelling information. Implementation of a time gap between LMWH administration and first administration of alteplase to avoid bleeding events on the first day of alteplase treatment with a combined short and long-term infusion.
Section to be changed		Section 4.2.1
Description of change 35		**Added**: Current or scheduled use of systemic (intravenous or oral) corticosteroid therapy should be included in SOC regimen (e.g. 6 mg of dexamethasone orally) for up to 10 days or until hospital discharge. This applies to both treatment arms. Total daily dose equivalencies to dexamethasone 6 mg are provided in Appendix 10.5. o Appendix 10.5 with proposed SOC therapies added o Recommendations before start of Alteplase treatment regarding insertion of arterial lines and central venous catheters, tubes, interventional procedures. o Management of bleeding and serious bleeding events recommendations during alteplase treatment period

Rationale for change		Prevention of bleeding events during alteplase treatment period; standardization of SOC therapy by providing guideline recommended treatment recommendations in Appendix 10.5
Section to be changed		Section 4.2.2.1 Restrictions regarding concomitant treatment
Description of change 36		**Added**: LMWH/unfractionated heparins in therapeutic dosages Patients who previously received a direct oral anticoagulant, can only be included, if the direct oral anticoagulant has been stopped. **Added:** for at least 48 hours prior to randomisation.
Rationale for change		Only prophylactic dosages of heparins allowed in combination with alteplase treatment for safety reasons. A time window of 48h after last DOAC dose is recommended to prevent overlap of therapies and with that risk of bleeding.
Section to be changed		Section 5.1.1
Description of change 37		Added: On Day 1 (randomisation day) an assessment just before randomisation shall be collected as a baseline value. The last assessment per day should be recorded in the case report form.
Rationale for change		Ensure standardization of data collection
Section to be changed		Table 5.1.1:1
Description of change 38		Mechanical ventilation ~~P~~PaO_2/FiO_2; corrected from <200 to <150; for Score 9
Rationale for change		Typo correction
Section to be changed		Section 5.1.2
Description of change 39		Added: oxygen free days
Rationale for change		To be aligned with endpoints collection
Section to be changed		Section 5.1.3
Description of change 40		Added wording: The qualifying PaO_2/FiO_2 ratio should be the worst measurement in a screening period. The worst measurement on a given day should be documented in the case report form.
Rationale for change		Ensure consistency of data capture between sites

Section to be changed		Section 5.1.4
Description of change 41		SOFA score evaluation timing added: If patient is discharged from hospital prematurely, the last evaluation needs to be performed at the day of discharge from hospital.
Rationale for change		Provide clear instructions to sites on expectations of last SOFA score evaluation
Section to be changed		Section 5.2.3
Description of change 42		**Removed wording**: Mandatory local laboratory parameters are: PaO2, haematology, ALT, AST, creatinine, potassium, sodium, LDH, CRP, fibrinogen, D-dimer and aPTT or anti-Xa. **Added wording:** More details on collection of safety laboratory parameters are summarized in Tab. 5.2.3:1
Rationale for change		Text abbreviation
Section to be changed		Section 5.2.6.1.3
Description of change 43		Updated wording for reporting of Always Serious AEs: For patients on the Alteplase treatment, these events should always be reported as serious adverse events, as described in Section 5.2.6.2. For patients on SOC, adverse events should be considered serious if they meet any of the seriousness criteria, outlined in Section 5.2.6.1.2
Rationale for change		The Always serious AE list is not applicable for patients on SOC as there is no requirement of expedited reporting of SAEs for the patients on SOC, as there is no IMP administered.
Section to be changed		Section 5.2.6.1.3
Description of change 44		For outcome events reporting added wording: Please refer to Section 5.2.6.2.4 for Exemptions to SAE reporting.
Rationale for change		Decision taken to exempt expedited SAE reporting of specific preferred terms (PTs), which are considered as progression of underlying disease.
Section to be changed		Section 5.2.6.2.1
Description of change 45		Added wording:

		The rules for Adverse Event Reporting exemptions still apply, please see Section 5.2.6.2.4.
Rationale for change		Decision taken to exempt expedited SAE reporting of specific preferred terms (PTs), which are considered as progression of underlying disease.
Section to be changed		Section 5.2.6.2.2.
Description of change 46		A**For patients not exposed to Alteplase treatment, but to SOC only:** No expedited reporting via SAE form is required. All safety data (AEs and SAEs) should be included in the eCRF.Consequently, the investigator is encouraged to report all adverse events for SOC group drugs in accordance to the local regulatory requirements for spontaneous AE reporting at the investigator's discretion by using their locally established routes and AE report forms. o For the patients on Alteplase treatment arm, the investigator must report serious adverse events, and non-serious adverse events which are relevant for the reported SAE on the BI SAE form
Rationale for change		There is no requirement of expedited SAE reporting for patients on SOC, as there is no BI IMP administered
Section to be changed		Section 5.2.6.2.3
Description of change 47		Added: A woman is considered of childbearing potential, i.e. fertile, following menarche and until becoming post-menopausal unless permanently sterile. Permanent sterilisation methods include hysterectomy, bilateral salpingectomy and bilateral oophorectomy. Tubal ligation is NOT a method of permanent sterilisation. A postmenopausal state is defined as no menses for 12 months without an alternative medical cause.
Rationale for change		Reflcting definition of women of childbearing potential and postmenopausal state
Section to be changed		Section 5.2.6.2.4
Description of change 48		Exemptions to SAE reporting: Disease progression of the underlying ARDS reflects the natural course of the disease, and is

		exempted from reporting as an (S)AE. Progression of the subject's underlying ARDS will be recorded on the appropriate pages of the (e)CRF only and will not be reported as an SAE. The specific (preferred terms) PTs that are exempt are: • Acute respiratory distress syndrome • Respiratory distress • Acute respiratory failure • Respiratory failure Death due to disease progression is also to be recorded on the appropriate eCRF page and not on the SAE form. Disease progression and death due to disease progression will therefore not be entered in the safety database and hence not get expeditiously reported. However, if there is evidence suggesting a causal relationship between the study drugs and the progression of the underlying ARDS, the event must be reported as SAE on the eCRF, as well as on the SAE form. Exempted events will be monitored at appropriate intervals by the external DMC.
Rationale for change		Decision taken to exempt expedited SAE reporting of specific preferred terms (PTs), which are considered as progression of underlying disease.
Section to be changed		Section 5.3.1
Description of change 49		Updated wording: As PK samples collection included less than 10 patients per treatment arm in Part 1, collection of PK samples will continue in Part 2. It is intended to obtain PK samples from at least 17 patients receiving Alteplase in Part 2.
Rationale for change		PK samples collection will continue in part 2 as there were not enough PK samples collected in part 1 of the trial.
Section to be changed		Section 6.2.1
Description of change 50		**Removed wording**: SARS-CoV-2 positive test result has to be available before treatment administration. **Updated wording** : For eligibility, patients should be hospitalized with the diagnosis of COVID-19 ARDS. SARS-CoV-2 laboratory confirmation (RT-PCR

		test) is a pre-requisite at screening. The RT-PCR test needs to be in timely association with the current hospital stay.
Rationale for change		More precise definition of eligibility and timely association of ARDS diagnosis and COVID-19 confirmed by RT-PCR.
Section to be changed		Section 6.2.2
Description of change 51		Updated wording: Randomisation of patients (Day 1, Visit 2a) shall happen not later than 24 hours after the confirmation of the inclusion and exclusion criteria. Study drug dispensing and administration shall start within 6 hours of randomisation. The time point of obtaining D-Dimer levels can be as long as two calendar days before randomisation.
Rationale for change		More clear wording on sequence of procedures and time window between screening and randomisation. Prolonged time point for D-dimer level collection for operational feasibility.
Section to be changed		Section 6.2.2
Description of change 52		**Added:** o Interruptions for urgent reasons for a total of 72 hours are allowed and accordingly, the time window of the visits may be extended. o wording for PaO_2/FiO_2 measurement: The worst measurement on a given day should be recorded in the CRF. o oxygen support is collected
Rationale for change		Added instructions on allowed treatment interruptions due to urgent safety reasons and timing of subsequent visits. Clear instructions for sites regarding which PaO_2/FiO_2 measurement shall be recorded in CRF. Oxygen support collection added for secondary endpoints assessment
Section to be changed		Section 7 Statistical methods and determination of sample size
Description of change 53		o Randomisation additionally stratified by D-Dimer status o Key secondary statistical objectives added o Confirmatory hierarchical testing procedure added to include testing of H0_1, Ho_2 and H0_3

		○ Analyses will be on the NIV patient cohort, and on the IMV patient cohort separately, as well as a potential a meta-analysis combining the NIV and IMV patient cohorts ○ Model adjustment covariates for the NIV patient cohort are the number of days under NIV support, baseline D-Dimer level and age. ○ Model adjustment covariates for the IMV patient cohort are the baseline WHO value, baseline D-Dimer level and age ○ Sensitivity analysis for primary endpoint on PPS. ○ Addition of ANCOVA for continuous secondary endpoints. Sample size rationale based on Part 1 results and changes to design including the introduction of the hierarchical testing.
Rationale for change		Based upon analysis at the end of Part 1, selection of dose for Part 2 and changes to design of study.
Section to be changed		Section 8.1
Description of change 54		Updated wording for PK sample collection in part 2: An optional assessment of pharmacokinetics also is planned to be performed in at least 10 patients per dose group in Part 1 and in approximately 17 patients in Part 2
Rationale for change		Updated number of PK patients in Part 2 reflecting number of collected PK samples in Part 1
Section to be changed		Section 8.4
Description of change 55		**Added:** The rules for Adverse Event Reporting exemptions still apply, please see Section 5.2.6.2.4.
Rationale for change		Reflecting update on exemptions for Adverse Event reporting
Section to be changed		Section 8.7
Description of change 56		**Added:** The DMC will also review at specified intervals the exempted events to SAE reporting as specified in Section 5.2.6.2.4.
Rationale for change		Update of DMC responsibilities reflecting update of exempted events to SAE reporting
Section to be changed		Section 9.1
Description of change 57		Added new references
Rationale for change		New references added in the Protocol
Section to be changed		Section 9.2
Description of change 58		Added unpublished reference

Rationale for change		Synoptic CTR part 1 completed
Section to be changed		Section 10 Appendices
Description of change 59		Appendix 10.1 Glasgow coma score removed
Rationale for change		Not planned to be used in Part 2
Section to be changed		Appendix 10.4 Monitoring
Description of change 60		**Added**: Due to the nature of this trial and the risks associated with Covid-19, site monitoring visits as physical on-site interaction may not always be possible. The focus will be on centralized oversight/monitoring. If not restricted by local Covid-19 regulations, we recommend combination of remote and on-site visits, mainly for initiation and close-out visits. Regular interim monitoring visits will occur mainly remotely unless the trial team requires a visit to be done on-site due to an identified risk where the required mitigation may need to be performed on-site. Trial monitoring intervals may be adjusted depending on enrolment rates, data quality, recruitment and conduct phases, timelines for CRF completion and/or site quality.
Rationale for change		Use of mixture of remote and on-site visits to increase quality of data collection.
Section to be changed		Appendix 10.4
Description of change 61		**Dosing regimen A (lower dose) dosing table (only applicable in Part 1 of the trial)** **Dosing regimen B (higher dose) dosing table (applicable in both Parts of the trial)**
Rationale for change		Reflecting decision on the trial – dosing regimen B for Part 2
Section to be changed		Appendix 10.5
Description of change 62		A list of guideline-recommended therapies for COVID-19 specifically, and for critically ill patients in general was added
Rationale for change		Standardization of SOC therapy across participating study sizes by providing guideline recommended treatment recommendations

11.3 GLOBAL AMENDMENT 3

Date of amendment		17 Feb 2022
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		The **TRISTARDS** trial - **T**h**R**ombolys**IS** **T**herapy for **ARDS** A Phase IIb/III operationally seamless, open-label, randomised, sequential, parallel-group adaptive study to evaluate the efficacy and safety of daily intravenous alteplase treatment given up to 5 days on top of standard of care (SOC) compared with SOC alone, in patients with acute respiratory distress syndrome (ARDS) triggered by COVID-19.
Global Amendment due to urgent safety reasons		
Global Amendment		**X**
Section to be changed		Protocol synopsis
Description of change		D-Dimer levels (≥3-fold to <5-fold ULN, versus ≥5-fold ULN) updated to Randomisation will be stratified by D-Dimer levels (≥ULN to <5-fold ULN, versus ≥5-fold ULN) updated from
Rationale for change		Stratification factor adapted based on the changed inclusion criterion of D-Dimer level ≥ ULN.
Section to be changed		Protocol synopsis
Description of change		Inclusion criteria #4 updated: Fibrinogen level ≥ **lower** limit of normal
Rationale for change		Alteplase results in a modest decrease of the circulating plasma fibrinogen levels. Fibrinogen levels ≥ ULN were previously required as an inclusion criterion to ensure that fibrinogen levels are high enough and are less likely to decreaseto a critical level below 150 mg/dL which have been

		associated with an elevated risk of bleeding events. A recommendation for temporary interruption of alteplase daily infusions if the fibrinogen level decreases to levels <150 mg/dL was implemented. The inclusion criterion of fibrinogen levels ≥ lower limit of normal is adequate in combination with the daily monitoring of fibrinogen levels and the implementation of a stopping rule for the alteplase administration if fibrinogen level decreases to levels <150 mg/dL.
Section to be changed		Protocol synopsis
Description of change		Inclusion criteria#5 update from: D-Dimer ≥ 3-fold of upper limit of normal (ULN) according to local laboratory to D-Dimer ≥ upper limit of normal (ULN) according to local laboratory
Rationale for change		Increased plasma D-dimers values are associated with an unfavorable prognosis in COVID-19. An increase in plasma D-dimers concentration with ongoing thromboprophylaxis should give consideration of the administration of higher doses of anticoagulants. The target patient population are patients with microvascular thombi. Hence, patients with suspected and confirmed pulmonary embolism are excluded from the trial. D-Dimer values ≥ ULN are more appropriate for a patient population with microvascular thombi and fibrin deposition within alveoli but without a high risk of thromboembolic events and with a high need anticoagulants in therapeutic dosages (which are restricted during the treatment period with alteplase).
Section to be changed		Protocol synopsis
Description of change		Exclusion criteria#1 updated: Massive, confirmed PE with haemodynamic instability at trial entry, or any (suspected or confirmed) PE that is expected to require therapeutic dosages of anticoagulants during the treatment period
Rationale for change		During the treatment period with Alteplase concomitant therapy with anticoagulants is allowed only in prophylactic dosages. The diagnosis of major thrombosembolic events such as pulmonary embolism requires therapeutic levels of anticoagulation. Therefore, patients with suspected or confirmed pulmonary embolism are excluded.

Section to be changed		Flowchart
Description of change		Dispense trial drug at V5/EOT removed
Rationale for change		Mistakenly ticked, no IMP dispense at V5
Section to be changed		Flowchart, Footnote 3
Description of change		The time-point of obtaining D-Dimer levels can be as long as **four** calendar days before screening. 2 days prior to randomization replaced by 4 days
Rationale for change		Increase flexibility for D-dimer level assessment to confirm eligibility of patients
Section to be changed		Flowchart, Footnote 7
Description of change		D-dimer levels shall be measured at Day 0 (or **no more than 4 calendar days before screening),** days 3, 5, 7 and 28
Rationale for change		Increase flexibility for D-dimer level assessment to confirm eligibility of patients
Section to be changed		Section 1
Description of change		Phase III corrected at different places to results of phase II
Rationale for change		Typos correction
Section to be changed		Section 1.1.
Description of change		Efficacy and safety results summary from part 1, phase II of the trial added
Rationale for change		Comprehensive results of safety and efficacy are provided based on the final data base lock to inform investigators and authorities.
Section to be changed		Section 3.1
Description of change		Randomisation in Part 2 will be 2:1 to dosing regimen B of alteplase (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 or to standard of care, and will be stratified by type of ventilation (non-invasive or invasive mechanical) and by **D-dimer levels (≥ULN to <5-fold ULN**, versus ≥5-fold ULN). D-dimer levels updated from (≥3-fold to <5-fold ULN, versus ≥5-fold ULN).
Rationale for change		Stratification factor adapted based on the changed inclusion criterion of D-Dimer level ≥ ULN.
Section to be changed		Section 3.3

Description of change		The time-point of obtaining D-Dimer levels can be as long as four calendar days before screening.
Rationale for change		To align with update in Flowchart
Section to be changed		Section 3.3.2 Inclusion criteria
Description of change		Inclusion criteria #4 updated to: Fibrinogen level ≥ **lower** limit of normal Inclusion criteria #5 updated to: D-Dimer ≥ upper limit of normal (ULN) according to local laboratory
Rationale for change		Please refer to changes to the protocol synopsis
Section to be changed		Section 3.3.3 Exclusion criteria
Description of change		Exclusion criteria #1updated to: Massive, confirmed PE with haemodynamic instability at trial entry, or any (suspected or confirmed) PE that is expected to require therapeutic dosages of anticoagulants during the treatment period
Rationale for change		Please refer to changes to the protocol synopsis
Section to be changed		Section 4.1.3 Method of assigning patients to treatment arms
Description of change		D-dimers adaptation included: In Part 1 the randomisation will be stratified by the type of ventilation support (invasive mechanical / non-invasive) and in Part 2 by both type of ventilation support and by baseline **D-dimer level (≥ULN to <5-fold ULN**, versus ≥5-fold ULN)
Rationale for change		Consistency with section 3.1
Section to be changed		Section 4.2.1 Other treatments and emergency procedures
Description of change		In Section Alteplase arm Day 1to 5 (treatment period) added recommendation for UFH: UFH dosage up to 5000 IU, SC BID (twice daily) can be given alternatively.
Rationale for change		Changes according to guideline on the use of anticoagulation for thromboprophylaxis in patients with COVID-19.
Section to be changed		Section 5.1.3 PaO_2/FiO_2 ratio
Description of change		Appendix 10.3 added: Fraction of Inspired Oxygen (FiO2) for common oxygen delivery systems are provided (Appendix

		10.3)
Rationale for change		Additional instruction for the estimation of FiO2 based on oxygen flow applied for common oxygen delivery systems provided.
Section to be changed		Section 7 Statistical Methods and Determination of Sample Size
Description of change		Updated wording for D-dimers: baseline D-Dimer level (≥ULN to <5 vs ≥5-fold ULN).
Rationale for change		Consistency with section 3.1 and 4.1.3
Section to be changed		Section 7.2.2 Primary Endpoint Analyses
Description of change		Added : Following the amendment to the inclusion criteria (CTP Version 4.0), whereby the original D-Dimer level changed from ≥3-ULN to ≥ULN, some additional analyses will be performed. Firstly, a sensitivity analysis will be added, similar to the primary analysis, just replacing the baseline D-Dimer status on the two levels with that on the three levels (≥ULN to <3, ≥3 to <5, ≥5-ULN). Secondly, a supplementary analysis will be added, similar to the primary analysis, but removing all patients with baseline D-dimer status ≥ULN to <3-ULN, which is according to the expanded inclusion criteria.
Rationale for change		Consistent with section 3.1, 4.1.3 and 7 The sensitivity and supplementary analyses have been added, to account for the change to the study population, and to adjust for or assess the impact of the changes.
Section to be changed		Section 7.4 Randomization
Description of change		Updated wording reflecting D-dimers update: Randomisation in Part 2 will be to alteplase and to standard of care, with an allocation ratio of 2:1, with stratification by the following factors: • Type of ventilation (non-invasive or invasive mechanical) • D-Dimer levels (≥ULN to <5-fold ULN, versus ≥5-fold ULN

Rationale for change		Reflecting update for D-dimer level requirements
Section to be changed		Section 8.3.2
Description of change		**Updated:** During the conduct of the study, it may not be possible for the investigator / institution to allow site trial-related monitoring, audits, IRB/EC review or regular inspections due to COVID-19 restrictions.
Rationale for change		Typo for IRB and added restrictions for COVID-19 for clarity
Section to be changed		Appendix 10.1
Description of change		Glasgow coma scale added
Rationale for change		Mistakenly removed in previous version
Section to be changed		Appendix 10.3
Description of change		Added Appendix: FiO_2 ranges for common oxygen delivery devices
Rationale for change		Additional instructions provided, consistency with Section 5.1.3
Section to be changed		Table10.6:1 Treatment Recommendations for COVID-19 ARDS (with a focus on NIV Patients)
Description of change		New references added, updated treatment recommendations for IL-6 inhibitors, JAK-inhibitors, oxygenation, ventilation and awakeprone positioning updated
Rationale for change		Updated based on up-to-date treatment recommendations

APPROVAL / SIGNATURE PAGE

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Signatures (obtained electronically)

Meaning of Signature	Signed by	Date Signed
Approval-Clinical Trial Leader	[redacted]	18 Feb 2022 09:24 CET
Approval-Team Member Medicine		18 Feb 2022 10:29 CET
Author-Statistician		18 Feb 2022 11:35 CET
Verification-Paper Signature Completion		18 Feb 2022 11:58 CET

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