COMBAT-ARF

Combat infectious ARF

EudraCT no. 2022-004079-17

"Efficacy and safety of 72-hour infusion of Prostacyclin (1 ng/kg/min) in mechanically ventilated patients with infectious pulmonary endotheliopathy – a multicentre randomized, placebo-controlled, blinded, investigator-initiated trial"

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Signature page

COMBAT-ARF trial

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"Efficacy and safety of 72-hour infusion of Prostacyclin (1 ng/kg/min) in patients with infectious respiratory endotheliopathy – a multicenter randomized, placebo-controlled, blinded, investigator-initiated trial"

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2. Medical emergency contact details

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3. List of abbreviations

AE	Adverse event
AR	Adverse reaction
ARF	Acute respiratory failure
ARDS	Acute respiratory distress syndrome
CRF	Case Report Form
GCP	Good clinical practice
hCG	Human chorionic gonadotropin
ICH	International conference on harmonization
ICU	Intensive care unit
ITT	Intension-to-treat analysis
i.v.	Intravenous
kPa	Kilopascal
MAP	Mean arterial blood pressure
MOF	Multi organ failure
NaCl	Sodium chloride
Ng	Nanogram
NYHA	New York Heart Association
PAH	Pulmonary arterial hypertension
PAOD	Peripheral arterial occlusive disease
PGI_2	Prostacyclin
PP	Per protocol analysis
QA	Quality assurance
RBC	Red blood cells
REDCap	Research Electronic Data Capture
SAE	Serious adverse event
SAR	Serious adverse reaction
SMS	Simplified Mortality Score
Sepsis	Presence (probable or documented) of infection together with systemic manifestations of infection
SmPC	Summary of product characteristics
sTM	Soluble thrombomodulin
SUSAR	Suspected unexpected serious adverse reaction
WHO	World Health Organisation

4. Protocol synopsis

Title	Efficacy and safety of 72-hour infusion of Prostacyclin (1 ng/kg/min) in mechanically ventilated patients with infectious respiratory endotheliopathy – a multicenter randomized, placebo-controlled, blinded, investigator-initiated trial
Brief title	COMBAT-ARF
Sponsor	Pär I. Johansson, MD, DMSc.
Clinical phase	Phase 2B
Trial type	Interventional
Purpose and rationale	The purpose of the trial is to investigate the efficacy and safety of continuous infusion of iloprost for 72 hours in mechanically ventilated patients with infectious pulmonary endotheliopathy
Trial design	This is a multicenter, randomized (1:1, active: placebo), blinded, parallel group trial with a total duration of day 90 follow up. Trial drug will be given as a 72-hours infusion of low-dose iloprost vs. placebo in addition to standard of care.
Trial duration	The trial will enroll patients over a 30-month period with a 90-day follow-up hereafter.
Primary Objective	The primary objective is to investigate whether continuous infusion of iloprost at a dose of 1 ng/kg/min for 72-hours reduces the 28-day mortality as compared to placebo.
Investigational drug and placebo	• Iloprost (Ilomedin®) • Saline (sodium chloride 0.9 %)
Population	A total of 450 adult mechanically ventilated patients with infectious pulmonary endotheliopathy
Inclusion criteria	• Adult intensive care patients (aged 18 years or above) • Suspected pulmonary infection • Need for mechanical ventilation (< 24hours from time of screening) • Endothelial biomarker (sTM) ≥ 4 ng/mL
Exclusion criteria	• Withdrawal from active therapy • Pregnancy (non-pregnancy confirmed by patient being postmenopausal (age 60 or above) or having a negative urine- or plasma-hCG) • Septic shock according to Sepsis-3 AND sTM>10 ng/ml • Known hypersensitivity to iloprost or to any of the other ingredients. • Previously included in this trial or a prostacyclin trial within 30 days • Life-threatening bleeding defined by the treating physician

	• Known severe heart failure (NYHA class IV) • Suspected acute coronary syndrome
Primary endpoint	• 28-day all cause mortality
Secondary endpoints	• 90-day mortality • Days alive without vasopressor in the ICU within 28-and 90 days • Days alive without mechanical ventilation in the ICU within 28 -and 90 days • Days without renal replacement in the ICU within 28-and 90 days • Numbers of serious adverse reactions within the first 7 days • Numbers of serious adverse events within the first 7 days
Methodology and statistical analysis	The analysis population will be defined as follows: *Intention-to-treat:* This will comprise all randomized patients except those who were randomised in error. This population will be evaluated for all endpoints *Per protocol* This will be the subset of trial participants who were correctly randomised, received the trial intervention according to protocol (i.e. 72-hours infusion of Iloprost or placebo after inclusion or until dead or discharge to ward, whichever comes first). This population will be evaluated for the primary endpoint only
Proposed start date	1. March 2024
Proposed end date	30 September 2026

5. Introduction

5.1 Background

The ongoing COVID-19 pandemic has highlighted the detrimental consequences of acute respiratory failure (ARF) on patient morbidity and mortality [1, 2]. ARF is common in critically ill patients and 50% of all intensive care unit patients require mechanical ventilation [3, 4]. ARF occurs in a heterogenous patient group, most often in the setting of pneumonia, sepsis, aspiration of gastric contents or severe trauma and major surgery [5, 6, 7]. Despite improvements in intensive care capabilities, ARF mortality remains high and the only treatment option, to date, is supportive care [8]. A recent Cochrane analysis (2018) found no evidence for that any drug was effective in reducing deaths in mechanically ventilated patients with ARF, highlighting the high unmet medical need [9].

COVID-19 research paves the way for novel treatment of ARDS patients

The COVID-19 pandemic revealed that infection-induced ARF is caused by injuries to the microvascular pulmonary endothelium [10]. The endothelium is a single cell layer that lines the innermost part of all vessels throughout the body [11]. The endothelium maintains the delicate balance between the circulating blood and the underlaying tissues that enables life and this interface maintain blood fluidity, transfers water and nutrients and immunity [11]. The damage to the pulmonary endothelium compromises the oxygen delivery from the pulmonary alveoli to the circulating blood [12]. Inadequate oxygenation of the blood (hypoxia) lead to organ failure in all vital organs including the heart, kidneys, liver and the brain, entitled multiorgan failure (MOF). More specifically we have reported that the level of circulating soluble thrombomodulin (sTM), which is a marker of endothelial damage, is strongly associated with organ failure and mortality in patients with systemic infections [13]. Importantly, Goshua and colleagues reported that COVID-19 patients with endotheliopathy defined by a soluble thrombomodulin (sTM) level above 4 ng/mL, reflecting severe endotheliopathy, had significantly increased mortality (40% vs. 20%) compared to those patients with sTM levels below 4 ng/mL [14]. Thrombomodulin is a key member of the anticoagulant protein C system and its cleavage from the endothelial surface contributes to the prothrombotic phenotype observed in mechanically ventilated COVID-19 patients with endotheliopathy [15, 16].

We have recently extended these findings beyond COVID-19 infection by studying 349 mechanically ventilated patients in a general intensive care setting [17]. Increased soluble TM was associated with a significantly increased 30-day mortality supporting the pivotal role of a prothrombotic phenotype for poor outcome in ARF. Similar to the findings in mechanically ventilated COVID-19 patients we found that also in the general intensive care patient requiring mechanical ventilation, those with sTM> 4 ng/mL had doubled mortality (38% vs. 19%) compared to patients with sTM below 4 ng/mL (Johansson PI et al. Manuscript currently in review). A pivotal finding here was that in patients with a respiratory infection as the cause of mechanical ventilation, the mortality in those with sTM> 4 ng/mL was 39% vs. 13% in the patients below this level, clearly pointing out the importance of pulmonary endotheliopathy for poor outcome.

5.2 Rationale for the trial

5.2.1 Prostacyclin – safety and effect

In 2010 the outcome of critically ill patients needing renal replacement therapy was studied at ICU 4131 at Rigshospitalet. We found that those receiving prostacyclin (PGI_2) as anticoagulant in the dialysis filter had substantially lower 30-day mortality than patients receiving heparin (21% vs. 39%), despite being more critically ill [18] and we speculated that this may be due to a spillover effect of PGI_2 to the systemic

circulation. PGI_2 is an endogenous prostanoid formed and released by endothelial cells with paracrine function including vasodilation and platelet inhibition. Because of these properties it was introduced as a pharmacological therapy in 1979 for patients with primary pulmonary hypertension and critical limb ischemia in high doses.

In the new millennium it was reported that PGI_2 also confers potent endothelial cytoprotection by: synthesizing endothelial glycocalyx constituents (hyaluronic acid) [19, 20], acting on prostaglandin I (IP_1) receptors on endothelial progenitor cells leading to re endothelium-formation in damaged vessels [21] upregulating VE-cadherin responsible for tight-junction integrity i.e. preventing capillary leakage [22], inducing peroxisome PPAR attenuation of NF-kB and TNF activation in ischemia-reperfusion injury which minimizes the inflammatory hit on the endothelium [23] and protecting against ischemia-reperfusion injury through the PGI_2-PPARα-HEME Oxygenase-1 signaling pathway that provide robust rejuvenation of the damage endothelium [24].

In a study in healthy volunteers, we demonstrated that low-dose PGI_2 (Iloprost) did not adversely affect blood pressure or platelet functionality but instead appeared to improve endothelial functionality as evaluated by soluble thrombomodulin (sTM) [EudraCT no: 2011-006200-12]. The effect of low-dose iloprost infusion (1 ng/kg/min) was, therefore investigated in randomized, double-blind pilot studies in patients undergoing major surgery (n=56) [25] and septic shock patients (n=18) [26]. These trials documented no adverse effect on blood pressure or platelet function. Instead, iloprost infusion significantly improved endothelial function and integrity, measured by validated biomarkers, in all groups. In septic shock patients we also found that sequential organ failure assessment (SOFA) score was significantly reduced together with reduced time on ventilator. In addition, a lower 30-day and 90-day mortality (8% vs. 34%; 25% vs. 50%) was demonstrated [26].

Proof of principle: The COMBAT COVID-19 trial

In the COMBAT COVID-19 trial we introduced a precision medicine approach by using the biomarker sTM at a level above 4 ng/mL as an inclusion criterion to ensure that only mechanically ventilated patients with severe endotheliopathy and high predicted mortality was included [27]. Eighty mechanically ventilated COVID-19 patients were randomized to 72-hours PGI_2 (Iloprost) 1 ng/kg/min infusion vs. placebo (41 Iloprost, 39 placebo) (NCT04420741). The primary endpoint was the median number of days alive without mechanical ventilation at 28-days, which was 16 days in the Iloprost group vs. 5 days in the placebo group (difference of the medians: 11 days [95% CI -5 to 21], P=0.07). The secondary endpoints included 28-day mortality, which was 21.9% versus 43.6% in the Iloprost and the placebo groups, respectively (RR% CI 0.24 to 0.96]). The incidence of serious adverse events <7 days were 2.4% vs. 12.8% (RR 0.19 [95% CI 0.001 to 1.11], P=0.10) in the Iloprost and the placebo groups, respectively.

5.3 Rational for investigational drugs

Investigators should be familiar with the product specifications for the investigational drugs.

5.3.1 Iloprost

Iloprost is a stable analogue of prostacyclin approved for marketing. Iloprost is approved in several countries in the EU as i.v. medication Ilomedin® for peripheal arterial occlusive disease (PAOD) and thrombangiitis obliterans and in Reynaud's disease unresponsive to other therapies. Iloprost is also approved as Ventavis® as inhaled medication for treatment of patients with pulmonary arterial hypertension (PAH), classified as NYHA functional class III.

5.3.2 Placebo (standard of care)
The placebo is saline 0.9 % (NaCl) to maintain blinding in the trial as iloprost is diluted in saline. Patients receiving placebo will receive an equal volume of fluid administered in the same way as the iloprost infusion.

5.4 Rationale for trial design
A phase 2 trial design is chosen to rapidly assess the safety and efficacy of Iloprost in patients with infectious pulmonary endotheliopathy to ensure rapid implementation.

6. Trial objectives

6.1 Hypothesis
Iloprost may be beneficial as an endothelial rescue treatment as it is anticipated to deactivate the endothelium and restore vascular integrity in mechanically ventilated patients with infectious pulmonary endotheliopathy, ultimately improving survival.

6.2 Objective
The main objective in this trial is to investigate whether continuous infusion of low-dose iloprost at a dose of 1 ng/kg/min for 72-hours is safe and significantly reduce mortality at 28 days compared to infusion of placebo in in mechanically ventilated patients with infectious pulmonary endotheliopathy.

7. Trial design
This is a multicenter, randomized (1:1, iloprost: placebo), placebo controlled, blinded, investigator-initiated in mechanically ventilated patients with suspected infectious pulmonary endotheliopathy, defined by circulating TM $\geq$ 4 ng/ml at the time of inclusion, investigating the efficacy and safety of continuous intravenous administrating of iloprost (1 ng/kg/min) vs. placebo for 72-hours, in a total of 450 patients. An independent DSMB will assess safety every for 100 patients included. The DSMB will revies 28-day mortality and other safety endpoints.

450 patients will be enrolled:

- Patients in the active treatment group (n = 225 patients) will receive iloprost infusion 1 ng/kg/min for 72 hours after inclusion, or until death or discharge to ward, whichever comes first.
- Patients in the Placebo group (n = 225 patients) will receive isotonic saline (equal volume) for 72 hours after inclusion, or until death or discharge to ward, whichever comes first.

Patients are presented at the investigator site in an acute critical condition and therefore informed consent will be obtained from a scientific guardian. Next-of-kin and subsequently the patient will co-sign as soon as possible (details described in *Section 14.2*).

During the trial additional blood samples will be taken at baseline and at 24-, 48- and 72 hours. Patients will be observed and assessed continuously. Patients will be actively assessed as long as he/she is in the ICU, for a maximum of 90 days. During the extended follow up period at day 90, data will be collected from department/hospital databases to establish potential mortality.

7.1 Endpoints

7.1.1 Primary endpoint

- 28-day all cause mortality

7.1.2 Secondary endpoints

- 90-day all cause mortality
- Days alive and vasopressor-free days in the ICU within 28- and 90 days
- Days alive and renal replacement-free days in the ICU within 28- and 90 days
- Days alive without mechanical ventilation in the ICU within 28- and 90 days
- Total number and numbers of patient with one or more serious adverse reactions within the first 7 days
- Total numbers and numbers of patients with one or more serious adverse events within the first 7 days (SAE is defined in section 12.3).

8. Patient selection, withdrawal and completion

The trial population is adult patients admitted to the ICU with infectious pulmonary endotheliopathy and need for intensive care. Patients will be considered eligible, if they comply with the inclusion and exclusion criteria below.

8.1 Inclusion criteria

1. Adult intensive care patients (age ≥ 18 years)
   AND
2. Suspected pulmonary infection
   AND
3. Need for mechanical ventilation (< 24 hours from time of screening)
   AND
4. sTM ≥ 4 ng/mL

To ensure that the sTM ≥ 4 ng/mL, the baseline sample collected at inclusion at the ICU are used. The sample must not be older than 2 hours before analysis.

8.2 Exclusion criteria

Patients are not eligible for inclusion in this trial if they fulfil one or more of the following criteria:

1. Withdrawal from active therapy
2. Pregnancy (non-pregnancy confirmed by patient having a negative urine- or plasma hCG or being postmenopausal defined as females at 60 years old or beyond or at the investigators discretion)
3. Septic shock according to the Sepsis 3 criteria AND a sTM> 10 ng/ml
4. Known hypersensitivity to iloprost or to any of the other ingredients
5. Previously included in this trial or a prostacyclin trial within 30 days
6. Life-threatening bleeding defined by the treating physician
7. Known severe heart failure (NYHA class IV)
8. Suspected acute coronary syndrome

NOTE: In patients with septic shock and sTM levels above 10 ng/ml no improvement in 28-day survival was observed in patients receiving low-dose prostacyclin for 72-hours in a recent randomized controlled study (Manuscript in development) and, therefore, the present study will exclude these from participating. Patients enrolled in other interventional trials will not be excluded unless the protocols of the two trials collide.

8.3 Discontinuation and withdrawal of trial intervention at the choice of the investigator

The investigator must discontinue study treatment for a given patient at any time for the following reasons:

- In case of intolerable serious adverse reactions/events, which are clinically relevant, suspected to be related to trial intervention and affects the patient´s safety. This will be at the discretion of the investigator.
- The patient is transferred to an ICU other than the trial sites

If the intervention is stopped due to a serious adverse event/reaction, the patient will be followed until the medical condition has been resolved.
The reason for discontinuation of study treatment will be documented in the patient CRF. Collection of trial related data will continue until day 90 and the participant will remain in the intention-to-treat population. This is outlined in *Appendix 1*.

8.4 Discontinuation and withdrawal of consent at the choice of the participant or the proxy

Participation in the trial is strictly voluntary. Patients, relatives or the scientific guardian can withdraw his/her consent at any time without giving further explanation, and without prejudice to further medical care and treatment.

If consent is withdrawn, the investigator will ask the participant or the proxy (if proxy-consent is given) to which extent the withdrawal includes to limit the amount of missing data. The withdrawal concerns either:

- Receiving further trial intervention only (allowing data registration and follow-up).
  OR
- Receiving further trial intervention AND further data registration and follow-up.

If withdrawal of consent to further trial treatment (meaning within the first 72 hours) the trial treatment is stopped, however the person withdrawing consent will be asked for permission to continue data collection from the patient medical record until day 90. If the patient or proxy withdraw their consent completely to further trial medication and data registration, no further data will be collected after this date. However, already collected data can still be used. The investigator must notify the sponsor immediately if a subject has been withdrawn. Outlined in *Appendix 1*.

8.5 Replacement of patients

A patient randomised in error (monitoring shows that in- or exclusion criteria have been violated) or who never received the correct trial medication, will be excluded from all data analysis, and replaced by randomising another patient.

8.6 Trial completion

Trial completion is defined as when the last patient completes their 90-day follow up

8.7 Trial discontinuation

The whole trial may be discontinued at the discretion of the sponsor in the event of any of the following:

- Interim analysis shows significant effects on mortality of the intervention
- Unexpectedly high rate of severe or life-threatening adverse reactions, which may indicate the premature closure of the trial (based upon the steering committee continual evaluation of SAR/SAE during the trial period)
- Medical or ethical reasons affecting the continued performance of the trial

In this case, sufficient measures will be taken to ensure patient interests. The sponsor will be responsible for informing the IEC and the Danish Medicines Agency within 15 days after termination of the trial.

9. Trial intervention

9.1. Identity of the investigational product

Iloprost (Ilomedin®) is a marketed product which will be administered in this trial. A description of it can be found in the Danish product specification (SmPC) for Ilomedin®. It will be handled as described.

9.1.1. Packaging and labeling of the investigational product

The drug used in this trial will be labeled according to local regulations.

9.1.2. Storage, issue, and return of investigational product

Iloprost is supplied by the Capital Region hospital pharmacy. A copy of a signed receipt will be kept in the trial site files. After the trial is completed, the investigators should be contacted to determine how to treat any leftover medication.

The investigators on each site will be responsible for the storage, dispensing, inventory, and accountability of the clinical supply. An accurate, timely record of the disposition of all clinical supplies must be maintained as described below:

- The identification of the patient to whom the drug was dispensed
- The date(s) and quantity of the drug dispensed to the patient
- The product batch number
- The product expire-date

Iloprost will be stored at each site at room temperature, separate from other medication. The preparation of the investigational drugs for each patient must be documented on a 'Drug Preparation and Dispensing Log Form' filed in a blinded site file, with restricted assess for unblinded personnel only.

9.2. Identity of Placebo

Saline 0.9 % (Sodium chloride®) is a marked product which will be used as placebo in this trial. Saline 0.9 %, 100 ml solution for infusion will be taken from ward supply at the ICU and will be handled as described in the Danish product specification (SmPC).

9.3 Preparation of investigational drug (active, placebo)

The trial drug will be prepared as described in the Danish SmPC, in brief described below. Individual patient- and center – drug use accountability records will be held according to GCP- recommendations.

The preparation will be done by an unblinded nurse at the respective ICU´s, who will be responsible for preparing the investigational drug so that it can be administered in blinded fashion. The preparation will be verified by double control.

9.3.1 Brief description of investigational drug preparation:

Iloprost is a colorless fluid that is to be diluted in 0.9% saline. The infusion pump containing diluted active drug and placebo will not be wrapped or sealed to hide the content as there is no difference between how the fluid looks and behaves.

The following dilutions are done and administered:

Weight (kg)	Infusion-rate (ml/h)	Iloprost vials	Dilution		Concentration (µg/ml)	Dilution Volume (ml)	24-hour infusion volume (ml)
			Iloprost (ml)	Saline (ml)			
30 – 39	3	2	4.1	100	0,778	104.1	72
40 – 49	3	3	5.2	100	0,980	105.2	72
50 – 59	3	3	6.3	100	1,185	106.3	72
60 - 69	3	3	7.4	100	1,378	107.4	72
70 – 79	3	4	8.6	100	1,575	108.6	72
80 – 89	3	4	9.8	100	1,785	109.8	72
90 – 99	3	5	11.0	100	1,982	111.0	72
≥100*	3	5	12.3	100	2,191	112.3	72

**Patients above 100 kg will all receive the 100 kg dose.*

9.3.2. Investigational drug: Iloprost

Delivered in 2,5 ml (20 µg/ml) vials.

Infusion: 4,1 – 12,3 ml iloprost (20 µg/ml) is diluted in a volume of 100 ml 0.9% saline and maximum concentration of 2,191 µg/ml for patients with a body weight from 100 kg at above. Administration of 1 (+ 0,30) ng/kg/min iloprost is attained with an infusion-rate of 3 ml/hour.

After dilution, iloprost can last for 24h meaning that an unblinded nurse from the respective ICU´s, must prepare the appropriate amount of the investigational drug (active, placebo) three times: Immediately after randomization (for infusion the first 24h) and again after 24h and 48h (for infusion the next 2x 24h).

9.3.3. Placebo
Volume of 72 ml isotonic saline per 24h. The precise volume and infusion rate to be administered to the patient is similar to the volume calculated for active drug and placebo.

9.4 Labeling of trial investigational drug (active, placebo)
After preparation/dilution of the investigational drug (active, placebo), a pre-formed label is put on the saline solution bagand filled out with preparation date and time, expiry time-point and, infusion rate and delivered at the ICU´s. The investigational drug/placebo will be administered to the patient by a nurse from the respective ICU´s. The label design will be as follows:

COMBAT-ARF trial

Investigational drug of 1 ng/kg/min Iloprost or placebo (saline)

Patient ID no.: _____

CPR: _ _ _ _ _ _- _ _ _ _

Volume: ___100_____ ml

Infusion rate: ____3____ ml/h

Expiry 24 hours after administration start (see patient CRF)

Preparation date: _____ Time: _____

Emergencies: Pär Johansson Phone: +45 2372 9202
For clinical trial use

9.5. Dosage and administration of investigational drug
All patients will receive 72-hour continuous infusion of either active investigational drug or placebo. Patients on active treatment will receive continuous infusion of 1.0 ng/kg/min iloprost. The infusion volume of the active investigational drug and placebo will be 72 ml per 24h.

9.6. Treatment compliance
Any reasons for non-compliance will also be documented.

9.7. Intervention Accountability
The trial site investigator is responsible for providing the necessary logistics for blinded investigational drug preparation at first knowledge of an incoming patient requiring mechanical ventilation due to suspected pulmonary infection. The investigational drug must be available when the patient fulfills the inclusion criteria:

- Performing stratified randomization as soon as the patients is found eligible
- Record the drug in the accountability log in the Drug dispension site file

- Immediate initiation of investigational drug infusion.
- Prepare and record new drug every 24 hours for a total of 72 hours

9.8. Randomization

The Sponsor is responsible for setting up the randomisation system. The randomisation sequence will be done in permuted blocks of variable sizes stratified for trial site using centralised, concealed allocation. The randomisation sequence will be generated 1:1 (active/placebo) using the online randomisation software 'Sealed Envelope' (https://www.sealedenvelope.com/). Once generated the randomisation sequence will be formatted and uploaded into REDCap to facilitate centralised, web-based allocation according to local written instruction. The randomisation sequence will be printed and signed by two independent individuals and stored in a sealed envelope in sponsors TMF.
The patient randomisation at each site will be done in the electronic system REDCap, where each patient will be given a unique randomisation number/Trial ID number. The randomization sequence will be concealed from all clinicians, patients, investigators and statisticians and will first be opened after completion of all trial related procedures and statistically analyses are finalised.

9.9. Emergency unblinding

Unblinding should only be undertaken is case of emergency when it is essential for the patient safety to get knowledge of the treatment assignment. Investigators can unblind the specific patient in REDCap, without knowledge of treatment allocation of the other patients. Investigator must inform sponsor immediately after any unblinding. An assessment will be done by the appropriate trial site personnel and the sponsor after an emergency unblinding to determine whether the investigational drug should be discontinued for a given patient.

10. Trial procedures

10.1 Patient eligibility

It will be the responsibility of the treating physician to identify eligible adult mechanically ventilated patients with pulmonary endotheliopathy due to a suspected respiratory infection and pass on information to the trial. All patients that fulfil inclusion criteria (described in section 8.1, bullet 1-3) are subjected to screening, which will be recorded on screening log. Patients fulfilling any of the exclusion criteria from the list described in Section 8.2 will not participate. The reasons for not entering the trial will be registered.

However, for all subject eligible for screening but excluded due to sTM ≤4 ng/mL the bloodsample taken as part of the screening process (baseline), will be stored in a research biobank. At the end of the study these samples will be transferred to a biobank for future research in acute critical illness (see Section 11.2.3). No clinical data will be collected from these patients, besides the information contained on the screeningslog (age, initials and sTM value). A separate informed consent will be collected from these patients (see Section 14.2).

If patients are deemed to be eligible for either screening only or inclusion in the study, consent for entry into the trial will be sought (see Section 14.2).

10.2. Schedule of intervention

The following procedure will be conducted after admission to the ICU.

Screening

- Assess eligibility (refer to inclusion/exclusion criteria, besides sTM biomarker)
- Informed consent from scientific guardian will be obtained if inclusion/exclusion criteria are fulfilled (besides sTM)
- Screening blood sample for endothel biomarker (sTM) will be analysed

Randomisation

- Patient is eligible for randomised if sTM ≥ 4 ng/ml
- Randomisation to Iloprost or NaCl

Baseline to 72 hours

- Administration of investigational drug every 24 hours
- Blood samples for measurement of endothelial biomarkers, mass spectrometry, and DNA analysis will be drawn at baseline and at 24, 48 -and 72 hours.

Baseline to day 90

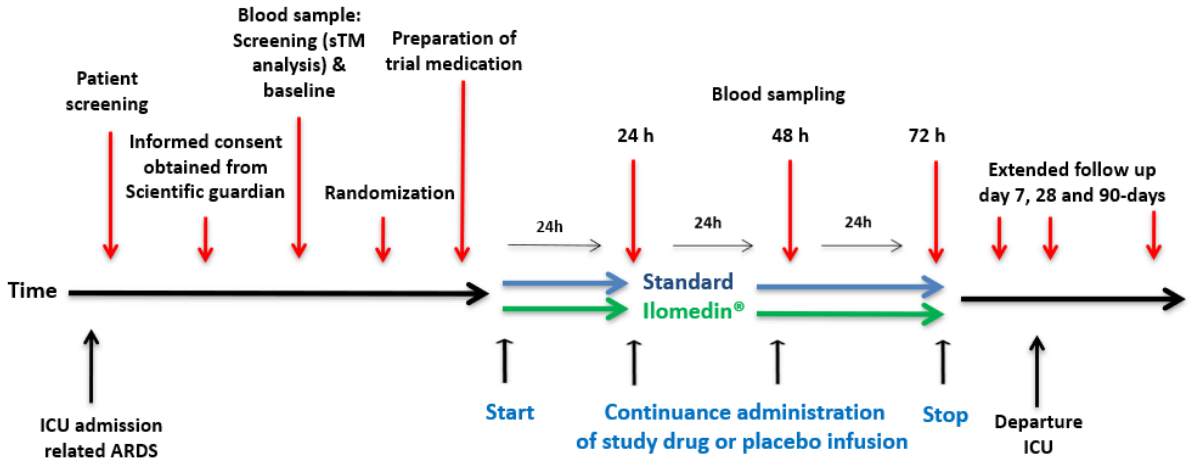
The following assessment will be recorded daily up to day 90 post baseline from the patients' medical journal

- Mechanical ventilation in the ICU (Yes/No)
- Vasopressor treatment in the ICU (Yes/No)
- Renal replacement therapy in the ICU (Yes/No)
- Serious adverse reactions (SARs) and serious adverse events (SAEs) until day 7
- Survival status day 28 and 90 (if death, date of death)
- Length of stay in the ICU
- Total length of stay in hospital

Note – Data will be collected until discharge from the ICU for maximum of 90 days after inclusion. Data on readmissions to the ICU during the 90-day period will NOT be included, besides in *total length of stay in hospital*.

However, if the patient is discharged before Day 7 from the ICU, the patient will be assessed for the occurrence of SARs/SAEs up to day 7.

10.3 Trial flow diagram



10.4 Trial table of observations and blood sampling

	Screen/ Baseline	24 h	48 h	72 h	Day 7	Day 28	Day 90
Informed consent - scientific guradian	x						
sTM analysis for inclusion	x						
Inclusion/Exclusion criteria	x						
Demographics	x						
Relevant medical history	x						
Pregnancy test (if neceasary)	x						
Randomisation	x						
Study blood samles	x	x	x	x			
DNA blod sample	x						
SAE/SAR		x	x	x	x		
Mortality						x	x

10.5 Recruitment period

First patient in: 1 March 2024
Last patient in: 30 September 2026

10.6 Number of patients

A total of 450 patients will be recruited in a 1:1 ratio (Iloprost: placebo).

11. Trial assessments

The following sections describe the methods of assessments and list the type of data to be recorded in the case report form (CRF).

11.1 Clinical assessments

11.1.1 Demographic data and medical history

The medical history (for details see below), including demographics, that will be collected from the patient medical journal are as listed below:

- Patient characteristics: Unic patient idenfifier, name, CPR. no, patient sex and age
- Medical history (prior assessment of a clinician during this admission), including any history of chronic cardiovascular disease, chronic lung disease, metastatic cancer, active haematological cancer
- Inclusion date and time

11.1.2 Disease severity

Raw data related to disease severity in the last 24 h prior to randomisation (use of mechanical ventilation, PaO_2/FiO_2, Platelets, Bilirubin, MAP, use of vasopressor type/dose, creatinine and urine output), for a maximum of 90 days during ICU stay.

11.1.2 Data from medical chart

- Clinical status
    - Mortality day 28 and 90, including date of death
    - Use of vasopressor, mechanical ventilation and renal replacement therapy in the ICU until day 90
- Length of stay in ICU and hospital
- SAE/SAE until day 7

11.2 Laboratory assessments

The normal procedures for sampling, handling, storage, and transfer of the laboratory samples will be followed for routine samples. The additional blood sampling for endothelial biomarkers, SNPs and plasma metabolomics will be obtained up to 72 hours after inclusion. All trial material such as test tubes and labels will be labelled with the patient randomisation number in addition to the routinely used information.

11.2.1 Biochemistry and haematology (routine samples)

Routine blood samples (~10 ml) will be drawn daily each morning during the ICU stay or for a maximum of 90 days.

These samples will be transferred to the local laboratory at trial site for analysis of the following parameters. The reference ranges used are those of the local laboratory at the trial site.

- Routine Laboratory Analyses: Haemoglobin and platelet count, bilirubin, creatinine. Sample for arterial blood gas (PaO2) is analysed in the ICU

11.2.2 Endothelial , plasma metabolomics and SNP analyses (additional study specific samples)

Additional blood samples will be drawn at baseline and 24, 48 and 72 hours after randomisation. A total of 20 ml of whole blood will be collected at each timepoint, meaning 80 ml in the whole trial period. All samples will be transferred to the Blood Bank, Capital Region for further processing (centrifugation, plasma and buffy coat isolation, aliquoting and freezing) to the research biobank.

Blood tubes required for the above analysis:

- 3 x 3 ml EDTA tube

- 3 x 3.5 ml citrate tube

The following markers are planned to be measured at baseline, and 24, 48, 72 hours after inclusion, at The Blood Bank Hemostasis Laboratory, 2034, Rigshospitalet, where sponsor is affiliated.:

- Endothelial biomarkers: syndecan-1, thrombomodulin, PECAM

The patients will be genotyped by the Global Screening Array (GSA) by Illumina. Only SNP´s related to genes coding for gene products involved in cell metabolism together with SNP´s related to genes coding for endothelial cell constituents will be used in this project. The DNA will be isolated from 3.5 mL citrate blood (at baseline only). The SNP analyses will be performed at the Institute of Molecular Medicine, Helsinki, Finland.

Also, Frozen plasma isolated from a 3 mL EDTA blood (at baseline and 24, 48, 72 hours) will be sent to MS-Omics, Vedbæk, Denmark. Here the plasma samples will be analyzed by mass spectrometry for metabolites. Any excess material after SNP- and mass spectrometry analyses has been carried out will be destroyed.

11.2.3 Research biobank and biobank for future research

Blood samples will after processing be stored in a temporary research biobank at the Haemostasis Research Laboratory in the Blood Bank at Rigshospitalet, Denmark. For laboratory quality reasons all samples are being analyzed at the end of the trial. The purpose of this research biobank is to examine what effects of Iloprost, when compared to placebo (standard of care) has on the vascular system (endothelium) and other aspects of the disease.

The research biobank will terminate latest on 31.12.2032. Any excess material will be stored in a biobank for future research in acute critical illness. Any remaining material can only be used in a new research project with a prior approval from a Research Ethics Committee. The biobank for future research will be reported to the local Data Protection Agency.

Blood samples from screening from patients not fulfilling the inclusion criteria sTM>4 ng/ml will be stored in the research biobank until end of the study. Hereafter, the samles will be transferred to the biobank for future research in acute critical illness with one of the purposes of performing a quality/validation study of a future point-of-care biomarker test for sTM. Theses participants will sign a separate consent form for the biobank for future research. Also, any remaining material from screening for patients included in the trial will also be used for validation of the sTM test after approvel by the authorities.

12. Safety recording

12.1 Definitions

Adverse Event (AE); is any untoward medical occurrence in a patient or clinical trial subject, administered a medicinal product and, which does not necessarily have a causal relationship with this treatment.

Adverse reaction (AR); is any untoward and unintended response in a patient/trial subject to an investigational medical product which is related to any dose administered to that patient.

Serious Adverse Event (SAE) or Reaction (SAR); any untoward medical event or reactions that at any dose results in death, is life-threatening, requires hospitalisation or prolongation of existing hospitalization, results in persistent or significant disability or incapacity or is a congenital anomaly or birth defect. The term "life-threatening" in the definition of "serious" 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 which hypothetically might have caused death if it were more severe.

Suspected Unexpected Serious Adverse Reaction (SUSAR); is a serious adverse reaction, where the nature and severity are not described in the Danish SmPC for Ilomedin®.

12.2 Risk and safety issues

Patients in this trial are present with respiratory failure. These patients will be admitted to the ICU due to a life-threatening condition challenged by failure of one or more organs and with a mortality above 40 % [27]. Many of these patients will have an underlying disease as well. Because of the patients' condition, many of these patients will during their treatment in the ICU be very dynamic and experience many clinical symptoms characterised as AE and SAEs during their stay. Almost all of these clinical symptoms and SAEs will be related to ARDS and concomitant underlying disease and not by related to the IMP. Recording of all SAEs in the CRF will not add valuable information to the patient's safety in this trial and will make it difficult be distinguish the real safety signal and those sight of the significant reactions. Therefor SAEs are not recorded as an entity. However, these patients are closely monitored at all times at the ICU and all AEs and SAEs are documented as part of standard practice in the patient electronic health record (i.e., ICU notes, laboratory reports); this will allow for later inspection if needed. Safety of these patients have high focus and all serious events that are not part of the expected course of this patient group will be evaluated on and ongoing basis in consultation with primary investigator, sub-investigators and sponsor. In addition, meeting is routinely held between PIs and sponsor. Also, a large number of significant safety parameters are recorded as endpoints for later safety and efficacy evaluation in each treatment group.

The investigator will record the occurrence of all SARs and those SAEs of special interest (listed in 12.3) until day 7 for all included patients in the electronic CRF. SAEs/SARs are only recorded until day 7 as no further safety concerns beyond day 7 is expected to due to the short half-life of the trial drug. Safety assessment will be done comparing safety events for iloprost versus placebo.

Patients will not be withdrawn from the trial if a SAR occurs, but it will be recorded in the CRF. For a detailed description of known seriously adverse reactions (as well as adverse reaction) for Iloprost; see section 4.8 in the Danish SmPC. The volume of 72 ml NaCl per 24 hours does not gives any safety concerns in this population.

12.3 SAEs of special interest

The following events are events of special interest as they either are relevant due to the pharmacokinetic of Ilomedin or of special concern in these patients as identified in the SmPC for Iloprost. Only events that fulfilled the serious criteria will be recorded. These SAEs are not subject to expedited reporting by the site to sponsor but must be recorded in the electronic CRF immediately after day 7, so that sponsor at any time have the possibility to monitor the occurrence of these SAEs and make a benefit/risks assessment of the trial.

- Ischaemic events (Cerebral (verified by CT), myocardial (STEMI and Non-STEMI), intestinal or limb ischaemia) [32]
- Bleeding events requiring more than 4 RBCs within 24 hours or ongoing bleeding
- Bleeding events (intracerebral haemorrhage (verified by CT) and lower gastrointestinal bleeding (defined as bloody diarrhea and, rectal bleeding)
- Severe cardiac failure (defined as severe cardiogenic shock and ejection fraction < 20 % cardiac ultrasound)
- Pulmonary embolism (symptomatic and verified by CT)
- Deep vein thrombosis (symptomatic and verified by ultrasound)

12.4 Recording of SAE/SAR

All SAEs listed in 12.3 are recorded in the electronic CRF until day 7.

Causal relationship to the IMP (Ilomedin®) will be judged by a medically qualified investigator upon his/her knowledge about the trial product, time-to-onset, underlying disease etc., according to the following definitions:

- Related: There is a reasonable relationship to the IMP
- Not related: There is probably no relationship to the IMP

If any SAE (including those listed in section 12.3) are judged as related to the IMP, the investigator must report these immediately to the sponsor, within 24 hours from nowing of the event.

The sponsor will then determine expectedness of all reported SARs according to the reference safety information contained in the Danish SmPC for Ilomedin. SUSARs are reported to the Danish authorities according to section 12.5.

In this trial, a pre-existing condition (i.e., a disorder present before the intervention has started) should not be reported as an SAE/SAR unless the condition worsens, or episodes increase in frequency during the reporting period.

12.5 Reporting requirement to authorities

The Sponsor will report SUSARs to the Danish Medicines Agency and Research Ethics Committee within 7 days for those that are fatal or life-threatening. All other SUSARs will be reported no later than 15 days from the time when the sponsor is informed. SUSARs will be reported in the EudraVigilance system.

Once a year, the Sponsor will submit a list of all SARs in the reporting period and a report on patient safety to the Danish Medicines Agency and Ethics committee.

The Sponsor will notify the Danish Medicines Agency and Ethics Committee when the trial has completed (no later than 90 days thereafter) and if earlier than planned due to safety, within 15 days with the reasons for stopping the trial. In addition, the results including endpoint, SAEs and SARs will be reported on EudraCT not later than 1 year after last patient last visit.

13. Analysis of trial data

13.1 Endpoints

13.1.1 Primary endpoints
28-day all cause mortality

13.1.2 Secondary endpoints

- 90-day all cause mortality
- Days alive without vasopressor in the ICU within 28 -and 90 days
- Days alive without mechanical ventilation in the ICU within 28 -and 90 days
- Days alive without renal replacement in the ICU within 28 -and 90 days
- Number of patients with 1 or more serious adverse reactions within the first day 7
- Number of patients with 1 or more serious adverse event within the first day 7

In addition, laboratory parameters (biochemistry and hematology) and vital signs will be summarized using descriptive statistics.

13.2 Definitions of evaluability

The definitions of trial populations are as follows:

Intention-to-treat:	This will comprise all randomized patients (except those randomised in error) [33] This population will be evaluated for all endpoints
Per -protocol	This is a subset of the intention-to-treat population encompassing correctly included patients who have received Iloprost or Placebo according to protocol (i.e. 72-hours infusion of Iloprost or placebo after inclusion or until dead or discharged to ward, whichever comes first). This population will be evaluated for the primary endpoint only.

Number of patients in and the available data for all three populations will be described in the required reports to the Danish Medicines Agency and Ethics Committee and in peer-reviewed scientific papers.

13.3 Statistical methods

Descriptive statistics will be calculated for all endpoints. All summary statistics of continuous variables will include n, mean, standard deviation, median, min/max and inter quartile ranges. All summary statistics of frequency tables will include counts and procentages. P-values <0.05 for the primary endpoint is considered significant.

13.3.1 Accountability procedure for missing data/population for analysis
If less than 5% of data are missing for any primary or secondary outcome, a complete case analysis without imputation of missing values will be performed. If more are missing all analyses will instead be based on the multiple imputations.

If multiple imputations are used, then the primary result of the trial will be based on these data. A simple complete case analysis will also be presented. If multiple imputation is used because of missing outcome data, we will performe a best-worst worst-best case scenario as a sensitivity analysis to. In the ‘best-worst-

case' scenario it is assumed that all participants lost to follow-up in the experimental group have had a beneficial outcome (e.g., have survived, had no SAE etc.); and all those with missing outcomes in the control group have had a harmful outcome (e.g. have not survived; have had a SAE etc.). Conversely, in the 'worst-best-case' scenario, it is assumed that all participants who were lost to follow up in the experimental group have had a harmful outcome; and that all those lost to follow-up in the control group have had a beneficial outcome. When continuous outcomes are used, a 'beneficial outcome' will be defined as the group mean plus two SD of the group mean or highest possible value whichever is smallest, and a 'harmful outcome' will be defined as the group mean minus two SD of the group mean or lowest possible value whichever is highest.

13.3.2 Primary endpoint

28-day all cause mortality will be compared in the ITT population using Fisher's exact test and effect size expressed as risk ratios with confidence intervals. All-cause mortality will be further illustrated using Kaplan-Meier curves.

13.3.3 Secondary endpoints

90-day mortality will be compared in the ITT population using Fisher's exact test and effect size expressed as risk ratios with confidence intervals and further illustrated using Kaplan-Meier curves. Other secondary endpoints will be compared using Wilcoxon test and differences expressed as changes in medians with non-parametric based bootstrapped 95% confidence interval.

13.3.4 Sample size and power

Patients will be recruited in a 1:1 ratio (Iloprost: Placebo). The number of patients participating is based on a power calculation using the 28-day mortality data in mechanically ventilated COVID-19 patients with sTM > 4 ng/mL from a randomized, double blind, placebo controlled clinical trial (NTC 04420741) showing a 50% reduction in the Iloprost arm.[27]. Assuming, conservatively, a 30% relative risk reduction in mortality in the Iloprost arm (from 39% to 26%) and providing the trial with 80% power to detect this difference at a significance level of 0.05 will require 203 patients per arm and 406 patients in total and to allow for a 10% drop out, 450 patients will be included.

13.4.5 Safety analysis (Performed by an independent Data Safety Monitoring Committee)

The independent DSMB will assess safety for every 100 patients included. The DSMB will review 28-day mortality and other safety endpoints. The DSMB decides its methodology and is free to ask to receive data in unblinded format. The DSMB will after each review provide a recommendation to the project steering committee. The DSMB can recommend one of the following three options: "Stop the study", "Pause the study" and "Continue the study". Accordingly, the safety analysis performed by the DSMB will not affect evaluation of the primary or secondary outcomes in the final analyses.

14. Ethical considerations

14.1 Trial Conduct

This trial is conducted in accordance with the Helsinki 2 declaration and ICH-GCP and in compliance with the protocol. The protocol, any amendments, the consent form, and the patient information must be approved by the health authorities (the Danish Medicines Agency) and by appropriately constituted independent Research Ethics Committee before trial initiation.

The trial is also reported to and approved by the Danish Data Protection Agency through the common application form of the Capital Region, Denmark. The trial complies with the Danish Health Act (Sundhedsloven), the General Data Protection Regulation (GDPR) and the Danish Act on Processing of Personal Data (Databeskyttelsesloven).

14.2 Patient information and informed consent

The informed consent procedure is conducted in accordance with Regulation (EU) No 536/2014, article 35 "Clinical trials in emergency situations". Informed consent will be obtained by a dedicated person selected by the primary investigator at each site.

Patients that are eligible for this trial will be temporarily incompetent due to acute severe illness relating to acute respiratory failure caused by suspected pulmonary infection and use of mechanical ventilation. To perform clinical trials with the goal of improving the treatment of respiratory failure for these patients, a life-threatening condition, it is necessary to include unconscious and incompetent patients as no clinically relevant animal model exists. There are no conscious patients that have the expected disease severity to benefit from an early treatment with the intervention suggested in this trial.

To make clinical trials with the goal of improving the treatment of critical ill patients, the treatment has to be initiated as early as possible where patients are temporarily incompetent. Therefore, informed consent is obtained after inclusion of the patient.

The Investigator or his/her qualified designee must as soon as possible after inclusion of the patient obtain written consent from the patient itself or proxy consent from both a scientific guardian and next-of-kin. Patients, who, during the course of this trial, become able to give consent, will be asked to participate and give their consent even though a proxy consent is obtained. The scientific guardian will be chosen based upon their independence from the trial and their knowledge of how respiratory failure is managed. The scientific guardian must, based on her/his knowledge of the research field, the basis of the trial protocol as well as the patients condition give concent to the trial (proxy consent). Each site will have specified a group of doctors to act as scientific guardians.

All types of consent can be captured on paper or by using a digital concent form in REDCap (a secure research electronic data capture system) where signatures will be written on a smart phone or tablet. The signed informed consent forms will be stored and available in REDCap, Region Hovedstaden. Informed consent will be paper based where digital consent is not possible. The informed consent forms must be signed and dated both by the patient or next of kin and by the person providing the information.

The next-of-kin and the patient will receive both written and oral information about the trial before giving consent. Both the written and oral information will be in an easily understandable language. The oral information can be supported be a video describing the project. This will align the information provided to the patients. Before signing the informed consent form, the patient or next of kin must be given sufficient time (i.e. > 24 hrs.) to read the study information and consider possible participation. Subsequently, they are informed that they are welcome to bring a "bisidder" when information about the study is provided. Participants are informed that it is voluntary to participate and that he/she can withdraw their consent to the study at any time without any consequences for their treatment, either now or in the future. The information to the patient or next of kin about the trial will be provided in a quiet undisturbed location. In those cases where informed consent is not obtained during the hospitalization, oral

information can be provided by telephone. Patients or relatives will receive a written participant information and a personal link using the REDCap solution to fill in consent via Digital Post (E-Boks) or obtained by regular post. Filling in consent from home, where the patient or relatives are not present to the person providing the information, requires login via NemID/MitID to verify person identity. Until a decision has been reached, the trail participant will continue to be include in the trial. Any attempt for obtaining consent will be documented in a log at each site.

Next-of-kin will be defined as; Closest relatives can be husband/wife, relatives in a straight line and siblings. Foster children will be considered as close relatives if they have regular contact to the subject. In cases where a subject does not have relatives in the form of a family member, or these cannot be contacted, other family members, friends and the like will also. with regular contact to the subject could be considered relatives. A concrete assessment will be made by the investigator.

14.3 Participant withdrawal/missing consent

In those cases where the next-of-kin or the patient does not consent, all trial-related procedures will stop. However, the data collected up to the timepoint of withdrawal can still be stored and used in the final database (see also section 8.4 regarding follow up for these patients).

Consent will always be tried to be obtained. However, in those cases where it´s not possible to obtain informed consent from either participant or next-fo-kin, e.g, no relatives or because relatives do not return and sign the consent form, patients stay incapaple to consent or the patient dies, one can subsequently record and use the necessary data from the patient's medical chart if approved by the ethical committee in the protocol. This also applies, if the patient dies before consent are obtained from both next-of-kin and the patient, data can still be used until time of death. However, in the event of death, a reasonable attempt will be made to obtain consent from next-of-kin hereafter either orally by phone or by letter (with the possibility of oral elaboration by phone or in person). Data can be obtained and use in the above-mentioned situations, if the participant is legally included in an emergency trial. However, if the trial participant does not become capable of consenting himself/herself within 90 days after inclusion, the consent from the trial participant or next-of-kin will no longer be sought.

14.4 Ethical justification

Participation in the COMBAT-ARF trial will not interfere with or delay diagnostic or therapeutic procedures necessary. Administration of investigational drug will not delay or interfere with therapeutic procedures or medication. Based on previous studies and the science literature, we expect that treatment with the active drug, iloprost, in the dose described here, will benefit the patients by improving endothelial function and potentially organ function. Furthermore, the dose that is used in this trial is considerably lower doses than normally used. Participation carries minimal risk for the patients as those with increased risk of side effects are excluded and there are strict stopping criteria if the risk of side effects increases during trial. Administration of investigational drug will not delay or interfere with diagnostic/therapeutic procedures or medication necessary.

- Knowledge of the endothelial protective effect of iloprost in patients with infectious ARF endotheliopathy cannot be gained outside the acute setting as proposed.
- The trial is being conducted to improve the treatment of patients with infectious ARF endotheliopathy, it is expected that the health of the trial subjects will improve in the long run.

- The interventions should be initiated immediately after screening and randomization, to alleviate the endotheliopathy. Therefore, consent from patients is not feasible, and awaiting the consent of relatives would induce an unacceptable delay institution of therapy in most cases.

- Administration of the investigational drug is expected to be of minimal risk to the patient and this is substantiated by data from two ongoing clinical trials in patients with septic shock and in patients with haemorrhagic shock secondary to trauma. A total of more than 400 patients have been included of whom 50% have received iloprost 1 ng/kg/min for up to 72-hours without serious adverse reactions have been registered.

- Increased knowledge of therapeutic potential of the intervention would increase the scientific knowledge of the condition of the individual and other patients with ARF endotheliopathy, without exposing the patients to high risk.

- Any relevant previously expressed objections to participation in clinical trials of the person known to the researcher will be respected, as will trial participation will be terminated by request of the next of kin.

- Inclusion in the trial may be of value to the individual patient but is valuable to the group of patients resuscitated from cardiac arrest in general, since further knowledge is needed to continue optimization of post resuscitation care.

- There are no consequences for the patient in connection with the SNP analysis mentioned in section 11.2.2 as no full genomic testing will be performed. Therefore, this analysis, cf. Section 7, 1 'Informationsbekendtgørelsen', has no consequences for the individual patient as we cannot identify any healt related issues out of the few very specific SNP markers which will be analysed.

15. Monitoring and quality assurance (QA)

15.1 Monitoring

The GCP-unit will carry out regular monitoring of this trial according to GCP. Monitoring visits to the trial sites will be made periodically during the trial according to the monitoring plan, to ensure compliance with GCP, the protocol and accuracy in relation to source data verification. Prior to inclusion of the first patient, investigative site personnel will document experience with GCP, and will receive appropriate training and instructions in the current protocol to enable trial conduct in accordance with GCP. Also, the trial site may be audited and inspected by the appropriate regulatory agencies. It is important that the Investigator and the relevant trial personnel are available during the monitoring visits and possible audits and that sufficient time is devoted to the process.

15.2 Access to data

The investigator or his/her delegates will collect relevant medical information from the patient medical chart to provide adequate health information needed to assess and evaluate each participant in the trial. The investigator has direct access to and guarantees direct access to source data/documents (including patient medical record) at monitoring, auditing and/or inspection visits by the GCP-unit and/or the Danish Medicines Agency. All data must be stored and kept confidential in accordance with the national legislations. All records are to be retained in a secure location for a minimum period of 25 years.

15.3 Data quality and data management

All collected data will be stored electronic in REDCap specially made for this trial. This Data magement system is secures, fully compliant with regulatory guidelines and a complete audit-trail for data entry validation. Trained staff at the sites will be responsible for data collection and entry into REDCap. The REDCap database will be set by sponsor at Righospitalet. During the study, the sponsor and sponsors management team will be in ongoing contact with the trial sites to ensure consistancy and good quality of the collected data. Sponsor will also perform regular data verification for data quality purpose and monitoring. For this purpose, the sponsor will have direct access to the patient's data and CPR no. in the REDCap database and Sundhedsplatformen.

15.4 Source data verification

Source Documents are original documents, records and data (e.g. hospital records, clinical and office charts, laboratory notes, memoranda, subject CRF and records kept at the pharmacy site file, recorded data from automated instruments etc.). Source Data are considered to be all information in original records and certified copies of clinical findings, observations, or other activities in the trial. Source Data are contained in Source Documents (original records or certified copies).
Most data will be entered into the CRF from patient files (source) by trial or clinical personnel under the supervision of the trial site investigators. When all patients have completed 90-day follow-up and data are collected, the database will be cleaned and locked. Statistical analysis and reporting will be done.
The location of source document will be registered on a form specifying where source data can be located e.g., medical record, CRF, lab reports etc. The investigator and delegated staff have direct access to source data (including patient files) for data verification and collection.

15.5 CRF handling

The main objective is to obtain those data required by the trial protocol in a complete, accurate, legible and timely fashion. CRFs are required and will be completed for each randomised subject. Data will be transferred to an electronic data capture system (REDCap). Source documentation supporting the CRF data will indicate the subject´s participation in the trial and document the dates and details of informed consent and trial procedures.

15.6 Changes to the final protocol

Any variation in procedure from that specified in the Final Trial Protocol may lead to the results of the trial being questioned and, in some cases, rejected. Any proposed protocol change will be documented in a protocol amendment, and this will be submitted to the Ethics Committee and the Regulatory authority for approval.

15.7 Deviations from the trial protocol

Deviations from the trial protocol, especially the prescription of doses not outlined in the trial protocol, other modes of administration, other indications, and longer treatment periods are not permissible (except in an emergency).

16. Finances

16.1 Finances

This research project is investigator-initiated by Pär I Johansson who also is sponsor of this trial. Funds to cover the trial related activities including salaries to assisting staff has been granted by a Novo Nordisk

Foundation grant of 7.0 million DKK and a Benzon Foundation grant of 4.1 million DKK to Prof. Johanssson. The amount is paid to a research account at Rigshospitalet and is administered by the economy department at Rigshospitalet. Novo Nordisk Foundation and Benzon Foundation has no influence on the design, the conduct or the results of the trial. The involved study sites and CAG Center for Endotheliomics at Rigshospitalet will support the trial. Neither patients nor health personnel will receive any remuneration from participating in the trial.

17. Insurance

The patients in the present trial are covered by the patient insurance, covering all treated patients at the trial sites ICU´s at Nordsjællands Hospital, Herlev Hospital, Bispebjerg Hospital and Sjællands Universitetshospital Køge in the event of a trial-related injury or death occurring.

18. Publication of trial results

The trial will be registered in the EudraCT database and on www.clinicaltrials.gov. Upon trial completion, the trial data will be made public, and manuscript(s) will be published in a peer review clinical journal regardless of whether the results of the trial were positive, negative or inconclusive. Authorship will be granted depending on personal input according to the Vancouver definitions. Data describing the trial design, safety and efficacy will be reported in EudraCT within 1 year after completion of the trial. The authors for the primary manuscript will be as follows: PSJ, , MB, NEC, LPKA, TL, JS, PIJ. After these authors, site investigators will appear as per the rules below (the order will depend on the number of included patients). Funding sources will be acknowledged, but they will have no influence on the data handling or analyses, the writing of the manuscript or the decision to publish. Secondary manuscripts may have other listing of authors as determined by the working group and according to the Vancouver definitions.

19. Trial organization

This trial is investigator-initiated by Pär I. Johansson, as collaborative research between the CAG Center for Endotheliomics at Rigshospitalet and Nordsjællands Hospital, Herlev Hospital, Bispebjerg hospital and Sjællands Universitetshospital I Køge. The trial sponsor is Prof. Pär I Johansson, MD, DMSC. (PIJ), coordinating investigator is Peter Søe-Jensen, MD, EDIC, (PSJ), Department of Intensive Care, Herlev Hospital and local investigators are Prof. Morten Bestle, MD, PhD, (MB) Department of Intensive Care, Nordsjællands Hospital, Niels E Clausen, MD, EDIC (NEC), Department of Intensive Care, Bispebjerg Hospital and Lars Peter Kloster Andersen, MD, PhD, (LPKA) Department of Intensive Care, Sjællands Universitetshospital.

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Appendix 1. Patient withdrawal/Discontinuation

Reason	To what	When	Consequent	Outcome	Data analysis	
					ITT	PP[2]
Withdrawal of consent	To further trial treatment	Within 72 hours[1]	Treatment is stopped	Patient is followed until day 90	X	-
		After 72 hours[1]	No consequent for study treatment	The patient is followed until day 90	X	X
	To further trial treatment **AND** data collection	Within 72 hours[1]	Treatment is stopped	Patient is **withdrawn**. No further data is collected	X	-
		After 72 hours[1]	No consequent for study treatment	Patient is **withdrawn**. No further data is collected	X	-
Patient transferred to	Ward	Within 72 hours[1]	Treatment is stopped	The patient is followed until day 90	X	X
		After 72 hours[1]	No consequent for study treatment	The patient is followed until day 90	X	X
	ICU other that trial site	Within 72 hours[1]	Treatment is stopped	Patient is followed until day 90	X	-
		After 72 hours[1]	No consequent for study treatment	The patient is followed until day 90 in the medical chart	X	X
Serious adverse reaction	*Related to* Ilomedin and affect patient safety	Within 72 hours[1]	Treatment is stopped	Patient is followed until day 90	X	-
		After 72 hours[1]	No consequent for study treatment	The patient is followed until day 90	X	X
Dead	-	Anytime from randomisation to day 90	-	The patient is NOT withdrawn. Day of dead is recorded.	X	X

[1] *Defined as hours after start of trial treatment*

[2] *Per protocol – Only patients who has completed the trial treatment will enter the per protocol analysis. This also includes those transferred to ward or who dies during the first 72 hours.*

Note: All randomized patients will enter the *intension-to-treat* analysis. However, subjects randomised in error will be excluded for all data analysis and will be replaced.