Therapeutic Iloprost for the treatment of acute respiratory distress syndrome (ARDS) (the ThIlo-Trial): a prospective, randomized, multicenter phase II study

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Study protocol

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Abstract

Background Acute respiratory distress syndrome (ARDS) is caused by rapid onset (within hours) acute inflammatory processes in lung tissue, and it is a life-threatening condition with high mortality. The treatment of ARDS to date is focused on the prevention of further iatrogenic damage of the lung rather than the treatment of the initial inflammatory process. Several preclinical studies have revealed a beneficial effect of iloprost on the control of pulmonary inflammation, and in a small number of ARDS patients, iloprost treatment resulted in improved oxygenation. Therefore, we plan to conduct a large multicenter trial to evaluate the effect of iloprost on ARDS.

Methods The Therapeutic Iloprost during ARDS (ThIlo-Trial) is a multicenter, randomized, clinical phase II trial assessing the efficacy of inhaled Iloprost for the prevention of the development and progression of ARDS in critically ill patients. One hundred fifty critically ill patients suffering from acute ARDS will be treated either by nebulized iloprost or NaCl 0.9% for 5 days. Blood samples will be drawn at defined time points to elucidate the serum levels of iloprost and inflammatory markers during treatment. Mechanical ventilation will be standardized. In follow-up visits at days 28 and 90 as well as 6 months after enrollment, functional status according to the Barthel Index, a health care-related questionnaire and frailty (Vulnerable Elderly Survey) will be evaluated. The primary endpoint is the improvement of oxygenation, defined as the ratio of PaO 2 /FiO 2 . Secondary endpoints include 90-day all-cause mortality, SOFA scores during the study period up to day 90, the duration of mechanical ventilation, the length of ICU stay, ventilator-associated pneumonia, delirium, ICU-acquired weakness and discharge localization. The study will be conducted in three university ARDS centers in Germany.

Discussion The results of the ThIlo-Trial will highlight the anti-inflammatory effect of iloprost on early inflammatory processes during ARDS, resulting in the improvement of outcome parameters in ARDS patients.

Background

Acute respiratory distress syndrome (ARDS) is defined as pulmonary compromise with bilateral pulmonary infiltrates associated with moderate to severe hypoxemia (1). The public health impact of ARDS is considerable, and it is estimated that approximately 75.000 cases of ARDS occur annually in Germany. The estimated mortality ranges from 26 to 51% and depends on the severity of the associated hypoxemia (2). Patients surviving ARDS treatment also show reduced functional capacity in their everyday life following hospitalization (3, 4). Therefore, there is a pressing need to develop further ARDS treatment strategies with a view to ultimately improving patient outcomes.

The bilateral pulmonary infiltrates that can be identified on chest radiography reflect the diffuse inflammatory changes within the lung that are caused by acute inflammation within the pulmonary tissue and the alveolar space. The initial inflammatory process is induced by the activation of the innate immune response by the binding of microbial products (pathogen-associated molecules [PAMPs]) or cell

injury–associated endogenous molecules (danger-associated molecular patterns [DAMPs]) to pattern recognition receptors (PRRs). Therefore, the common causes of ARDS are trauma, sepsis, pneumonia, blood transfusion or aspiration into the lungs. After the initial activation of the innate immune response, innate immune effector mechanisms, such as the formation of neutrophil extracellular traps (NETs), are activated, which further aggravate the alveolar injury (5). The resulting increased permeability of the microvascular barrier results in the extravascular accumulation of protein-rich fluid that accumulates within the alveolar space. The increased permeability is also linked to the transfer of leukocytes (mostly neutrophil granulocytes) and erythrocytes into the alveolar space in ARDS, as well as to the presence of proinflammatory-regulated cytokines that increase the inflammatory burden within the lung (5). As a result, dysregulated inflammation, the accumulation of leukocytes and platelets and altered permeability of alveolar barriers remain the central pathophysiologic problems in ARDS (5, 6).

The treatment of ARDS to date is focused on the prevention of further iatrogenic damage of the lung through lung-protective mechanical ventilation, neuromuscular blockade and conservative fluid management (7). Recent clinical trials have focused on the role of ventilation strategies in the prevention or treatment of ARDS using noninvasive ventilation devices or prone positioning (8, 9). Although these strategies showed a positive effect on patient oxygenation and symptoms, they do not interfere with the underlying pathophysiological changes of ARDS. Several interventions have tried to use a potential anti-inflammatory strategy for the treatment of the existing intra-alveolar inflammation or to intervene in the development of intra-alveolar inflammation. For this, patients were treated with aspirin, simvastatin and surfactant, but the tested treatments failed and did not have any significant effect (10-12).

Iloprost. There is a significant amount of evidence in preclinical models that show that the use of iloprost for the treatment of ARDS and pulmonary inflammation might be of significant benefit. In small animal models, investigators showed that iloprost improves endothelial barrier function and reduces the detrimental signs of pulmonary edema (13). It also reduces the pulmonary sequestration of leukocytes and platelets, which is a central disease mechanism underlying the development of ARDS (14). This evidence could be transferred into different models of lung injury, showing positive evidence for the reduction of pulmonary inflammation in a pressure-induced model of lung injury (15). The anti-inflammatory effect was attributed to the COX-2 system and the involvement of lipoxin A4 (16). RAP-1 might also be involved in the protective role of iloprost (17). This positive anti-inflammatory effect of iloprost on the pulmonary tissue was also demonstrated in several models of ischemia-reperfusion injury (IR). Furthermore, IR can also result in ARDS and pulmonary failure. Iloprost was able to reduce this pulmonary compromise in several preclinical studies (18-21). The anti-inflammatory effect of iloprost was also shown in large animal models of lung injury using porcine models of ARDS (22-24). Here, again, iloprost showed an anti-inflammatory effect. In addition, the shunt fraction could be reduced, which resulted in improved oxygenation and improved pulmonary dynamics, which is essential for the reinstitution of spontaneous ventilation during and following ARDS (22, 23, 25-27). This shows that the preclinical data identified a beneficial effect of iloprost on ARDS. So far, only one study on inhaled iloprost in adult ARDS patients has been conducted, although an application of inhaled iloprost is noted in the guidelines of the Association of Medical Scientific Societies (AWMF) for the treatment of ARDS

(28). The AWMF guidelines indicate that the use of ARDS can be considered, especially in patients with severe ARDS who are mechanically ventilated and not self-consenting (7).

Methods/design

Study design. ThIlo is a multicenter, randomized, single blinded clinical phase II trial assessing the efficacy of inhaled iloprost in the development and progression of ARDS in critically ill patients. Based on the risk of pulmonary hemorrhage which is very rare especially in patients with ARDS, study medication was unblended. For safety reasons, after treatment of 100 patients (day 28 after last dose IMP Patient 100) within the study an interim analysis for an increased risk for pulmonary hemorrhage ≥°III according to CTCAE Version 5.0 in the treatment (Iloprost) arm will be performed and the results discussed with the DSMB. It was approved by the local ethics committee (University Tuebingen, Germany) on June 4th 2019 and by BfArM (Bundesinstitut für Arzneimittel und Medizinprodukte, Bonn, Germany (EU/1/03/255/001)) on the 14th of March 2019. The trial is registered at EUDRA-CT (2016-003168-37) and at clinicaltrails.gov (NCT03111212).

Population. The target population for this clinical trial is adult critically ill patients suffering from ARDS. Patients will be included in the trial if they present with ARDS as defined by the Berlin Definition (Table 1 and (1)) and meet the inclusion criteria. The trial population will consist of both sexes. One hundred fifty intensive care patients suffering from ARDS will be included in the study at the Department of Anesthesiology, Eberhard Karls University Tübingen, Germany; the Department of Intensive Care and Intermediate Care, University Hospital RWTH Aachen, Germany; and the Department of Anesthesiology, University Hospital Münster (UKM), Münster, Germany.

Inclusion Criteria. Patients meeting the following criteria will be included: age ≥ 18 years, PaO_2/FiO_2 ≤ 300, bilateral infiltrates consistent with pulmonary edema on frontal chest radiograph, need for positive pressure ventilation via an endotracheal tube or noninvasive ventilation and no clinical signs of left atrial hypertension detected via echocardiography, or if measured, a Pulmonary Arterial Wedge Pressure (PAOP) less than or equal to 18 mmHg. The term “acute onset” is defined as follows: the durations of the hypoxemia criterion and the chest radiograph criterion must be ≤ 48 hours at the time of randomization. Patients must be enrolled within 48 hours of ARDS onset and no later than 7 days from the initiation of mechanical ventilation.

Exclusion criteria. The exclusion criteria are defined as follows: subject age < 18 years; time interval more than 7 days since the initiation of mechanical ventilation; more than 48 hours since the onset of ARDS; patient, surrogate or physician not committed to full intensive care support; positive pregnancy test at the time of screening; and contraindications against iloprost. These are defined as conditions in which the effects of iloprost on platelets might increase the risk of hemorrhage (e.g., active peptic ulcers, trauma, intracranial hemorrhage), severe coronary heart disease, myocardial infarction (within the last 6 months), decompensated heart failure, severe arrhythmias, unstable angina pectoris, pulmonary arterial hypertension caused by the occlusion of pulmonary veins, cerebrovascular events (e.g., transient

ischemic attack, stroke) within the last 3 months, and congenital or acquired valvular defects with clinically relevant myocardial function disorders not related to pulmonary hypertension. Patients who received iloprost treatment for any indication within 48 hours prior to enrolment in the clinical trial or patients who were on thrombin inhibitors or NO within the previous 24 h before study randomization were also excluded. Additionally, patients dependent on the sponsor, investigator and their employees were not included in the study.

Study drug. The investigational medicinal product is iloprost (Ventavis ®; Drug Code: SUB14185MIG; ATC code: B01AC11), manufactured by Berlimed S.A., Spain (for Bayer Pharma AG, Germany); it will be used as a concentrate for use in nebulizers and will be administered by inhalation three times a day (20 µg per administration). The administration of the drug will occur at the same time each day ± one hour. In cases of severe adverse effects, the dosage will be reduced to 20 µg once a day (morning). Other dose modifications or temporary cessation of the study drug will not be allowed.

Iloprost is usually dissolved in 0.9% NaCl, which is used to keep the ventilator circuit moist as standard of care. Therefore, in the control group, NaCl 0.9% will be used to keep the airway circuit moist, which is the standard of care for the treatment of patients with pulmonary insufficiency (7). Looking at the pharmacokinetic and dynamic profile of iloprost, we have suggested an approach of an application of three times per day, with a dose of 20 µg, which seems to be an average dose in the trials reported up to date. The rationale behind this was that iloprost also exerts an anti-inflammatory effect that may last up to 6 h (29-33). Therefore, an administration of iloprost 3 times a day would allow a significant time frame per day to be covered by anti-inflammation due to this drug. The duration of 5 days was included in the trial because the pathophysiology of ARDS develops within the first few days and is progressive during that period.

Randomization. Randomization lists will be generated at the biostatistical center. Based on these lists, numbered envelopes will be provided and used for randomization.

Concomitant Medication and Treatments

Relevant additional medications and treatments such as vasopressors, inotropes, anti-infective agents, inhalative therapy or sedation, steroids and immunosuppressive therapy administered to the subjects on entry to the trial or at any time during the trial are regarded as concomitant medications and treatments and must be documented on the appropriate pages of the CRF, these data will be grouped according to class of medication. Depending on the substance the documentation varies in details (e.g. dosing).

Intervention plan. This study will consist of the following consecutive phases: study entry, treatment and follow-up. The time-points and trial procedures are listed in Table 2. All patients included in this trial will receive standard care for ARDS according to the ARDS network, with special consideration of lung protective ventilation strategies.

Study entry

In this trial, ARDS patients present an emergency situation, such as the diagnosis of ARDS requiring ICU admission and ventilation therapy, not allowing for any delay of diagnostic work-up or therapy. Additionally, due to severe symptoms, the vast majority of patients who meet the eligibility criteria for the trial are assumed to be unable to give consent in the acute admission phase and legally authorized representatives (LAR) might not be available in most cases. This is also in line with local regulations: e.g. §41 of the German Drug Law allows the start of a treatment in an emergency situation without prior consent in case the immediate treatment is necessary to save the patient's life, recover the patient's health or ease the patient's suffering. In this situation the consent of an independent physician not directly involved in the study conduct will be sought before the beginning of any study-related activity. The consent has to be obtained as soon as the patient is able to give consent or a LAR is available. Independently, the personal consent will be obtained from each patient after recovering consciousness and competence for decision making or by a legal representative in cases recovering is not achieved during study duration (i.e., day 27). When possible however the patient or his legal representative is to be informed both in writing and verbally by the investigator before any study-specific procedure is performed. Each patient or his legal representative will be informed about the modalities of the clinical study in accordance with the provided patient information. Informed consent from the patient will be obtained using a form approved by the Ethics committee (EC) of the Universitätsklinikum Tübingen or the local EC if the patient is treated in a collaborating institution.

Treatment phase

The treatment group will receive 20 µg of nebulized iloprost three times per day for 5 days in addition to standard care. Iloprost will be measured in blood samples to determine the serum levels within this setting.

The control group will receive nebulized 0.9% sodium chloride (NaCl) with an equal volume three times per day for 5 days. After 5 days, the trial treatment will be complete (Figure 1). Blood samples will be drawn at defined time points for a variety of biomarkers to better assess the associations among coagulation, inflammation and iloprost treatment. Key cointerventions (infection control, aspiration precautions, fluid and transfusion) will be standardized across all patients. Mechanical ventilation will be standardized (see supplemental file).

Follow-up

Hospital survivors will undergo a brief follow-up phone survey to assess functional status (Barthel Index), a health-related questionnaire, and frailty (VES) six months after enrollment. The patients will be visited daily until day 28 or until discharge from the ICU, which could be beyond day 28. If discharged, the next visit will be on day 90, if patients are still in the ICU, there will still be daily visits until this time point. Data will be collected according to the study procedure until then. Each visit will consist of a clinical examination, a blood sample, assessment of the functional capacity through the Barthel Index and assessment of the severity of illness through the SOFA score. All data will be recorded on an eCRF form; this will be used as a visit diary. Blood samples will be drawn at defined visits for a variety of biomarkers to better assess the associations among coagulation, inflammation and iloprost treatment (Table 3).

Outcome measurements

Study Objectives

The primary objective and endpoint is to assess the effect of iloprost on the improvement of oxygenation (PaO_2/FiO_2 ratio) in patients suffering from ARDS.

For secondary objectives the absolute incidence of the following parameters will be determined:

- Overall survival in the 90-day follow-up period (90-day all-cause mortality).
- Duration of mechanical ventilation support
- ICU length of stay
- Ventilator-associated pneumonia
- Pulmonary hemorrhage
- Gastrointestinal hemorrhage
- Pulmonary embolism
- Delirium
- ICU-acquired weakness
- Discharge location (home, skilled nursing facility, rehabilitation)

The exploratory objectives are 6-month survival, QOL (SF12), functional status (Barthel Index), and frailty (VES) assessed by phone follow-up interview.

Efficacy Parameters

The following parameters will be used to determine the efficacy:

- Improvement of oxygenation (PaO_2/FiO_2) on a daily basis in relationship to baseline
- Overall survival in the 90-day follow-up period
- Decrease in duration and severity of ARDS
    - SOFA scores: will be calculated based on data in hospital records

    - Duration of mechanical ventilation support: documentation in hospital records
    - ICU length of stay: documentation in hospital records
    - Ventilator-associated pneumonia: documentation of microbiological findings in hospital records
    - Incidence of barotrauma: documentation of ventilator parameters in hospital records
- Reduced morbidity assessed through SOFA score, also according to the incidence of complications and increased functionality assessed through the Barthel Index
    - Delirium: documentation (e.g., CAM-ICU) in hospital records
    - ICU-acquired weakness: documentation in hospital records
    - Discharge location: documentation in hospital records, phone call

The demographic parameters at enrolment include age, sex, race, ICU admission diagnosis and comorbidities (such as diabetes, existing malignancy, any kind of pre-existing pulmonary disease, and hypertension).

The main clinical data during the ICU daily assessment are as follows:

- Laboratory data: Blood count, procalcitonin, IL-6, creatinine, urea, PTT, D-dimers, INR, AST, ALT, albumin, CHE, BNP
- Ventilation support
    - Invasive or noninvasive ventilation
    - Prone positioning Yes/No
    - Max P_{max} on daily basis
    - max P_{mean} on daily basis
    - PEEP on daily basis
    - PEEP on daily basis
    - Driving pressure at max P_{max}
    - compliance on daily basis
    - FiO_2 on daily basis
    - High frequent ventilation Yes/No
    - Tracheotomy Yes/No
    - Any relaxation therapy performed Yes/no
-
- ECMO therapy: Cannulation, blood flow, FiO_2, Sweep gas
- Volume resuscitation: Volume crystalloid/day, volume colloid/day, volume albumin/day, daily balance sheet
- Transfusion: Units red blood cells/day; units thrombocytes/day

- Substitution of clotting factors if necessary: Fibrinogen, PPSB, factor XIII, cyclocaprone, vWF, factor VIII, factor VII
- Anticoagulation: Fractionated heparin (cum. dose/day), unfractionated heparin (cum. dose/day), epoprostenolum (cum. dose/day), argatroban (cum. dose/day), others (cum. dose/day)
- Hemodynamic parameters: Heart rate, arrhythmia, lowest and highest MAP per day, lowest and highest ZVD per day, cardiac index, lowest and highest SpO_2 per day, lowest and highest lactate per day (mmol/l) SvO_2,
- Renal replacement therapy: AKIN criteria. CRRT, CVVHD, CVVHDF, CVVHF, intermittent dialysis, SLED/dialysis, anticoagulation, duration, dose
- Delirium: CAM-ICU score
- Immunosuppressive therapy: Yes/no

Weekly assessments of the ICU will include the following:

- Differential blood count
- Creatinine clearance
- ECMO post oxygenator PaO_2
- SOFA score
- Assessment at discharge
- Chronic renal failure at discharge
- Hepatic failure at discharge
- Length of stay in the ICU
- Length of stay in the hospital
- Discharge from hospital to a nursing home
- Discharge from hospital to home
- Discharge from hospital to a rehabilitation unit
- Residence in nursing home at 6 months

The final assessment will consist of the following:

- Days of ECMO support
- Ventilator days
- Tracheotomy
- Need for mechanical ventilation at home
- Incidence of pulmonary hemorrhage defined by an indication for blood transfusion, radiological finding, or a decrease in oxygenation
- Incidence of barotrauma

- Incidence of pleural drainage
- Incidence of pulmonary embolism defined by the following parameters:
    - New hypotension
    - Sign of right ventricular failure on echocardiography
    - Biomarkers
    - CT-scan (optional)
- Incidence of gastrointestinal bleeding defined by the following parameters: upper gastrointestinal bleeding, blood vomiting, lower gastrointestinal bleeding, melena, indication for blood transfusion, endoscopic diagnosis/intervention
- Incidence of cerebral hemorrhage defined by following parameters: Impairment as measured by the Glasgow coma scale, CT scan
- Infections: Incidence of positive blood culture, incidence of pneumonia, incidence of wound infection, incidence of peritonitis, incidence of surgical intervention due to infection, incidence of bacterial infection, incidence of fungal infection, incidence of viral infection, incidence of MRGN infection
- Anti-infective therapy: Generic, duration, incidence of changing antiinfective therapy due to inadequate treatment
- Incidence of surgical intervention

Data collection and management

Case Report Form

The trial case report form (CRF) is the primary data collection instrument for the trial. For this project, electronic Case Report Forms (eCRFs) will be used. Entered data will be subjected to plausibility checks directly implemented in the CRF, monitoring and medical review. The trial master file, the CRFs, and other material supplied for the conduct of the study will be retained by the sponsor/CRO according to applicable regulations and laws. The investigator(s) will archive all trial data (source data and Investigator Site File (ISF), including the subject identification list and relevant correspondence) according to the ICH Consolidated Guideline on GCP and local laws or regulations.

Statistical Analyses

Study Population Definition

The study population will consist of the following: to be assessed for eligibility: (n = 300); to be assigned to the trial: (n = 150); to be analyzed: (n=150 in the intention to treat analysis, other endpoints n=120). The sample size and power consideration refers to 120 evaluable patients, and it is assumed that the power will not be decreased in the analysis of the ITT population using multiple imputation. Furthermore, baseline adjustment will not be taken into account, which leads to a conservative sample size estimation.

In a previous study on iloprost with 20 patients, an increase from 177±60 to 213±67 was observed for the PaO_2/FiO_2, which was significant at the 0.01 level (27). Recalculation shows that the intraindividual standard deviation must have been considerably smaller as a p-value of 0.01 corresponds to an effect size of 0.93 (intraindividually) and thus to an intraindividual standard deviation of approximately 40 in this study.

In our study, we can show effect sizes of 0.525 assuming 116 error degrees of freedom, taking into account 1 day for baseline adjustment and 3 days for the study center (inquiry, power 80%, level of significance 0.05 two-sided, t-test). If we assume the recalculated standard deviation from the previous study in our study (which is still conservative due to the linear baseline adjustment used in our study), an (interindividual) effect size of 0.525 corresponds to a difference of approximately 21 in the PaO_2/FiO_2 ratio in the treatment arm compared to the control arm. This seems to be a reasonable and relevant effect.

Analysis of Primary Variables

The primary endpoint of PaO_2/FiO_2 at day 6 after the baseline will be analyzed daily using a baseline adjusted analysis of covariance model with the last measurement of the PaO_2/FiO_2 ratio before treatment as the baseline, with the study arm as a second level factor. The study center will be included in the analysis as a nuisance factor. Additionally, an interaction term between baseline and treatment will be included in the model if this term is significant. In the case of interaction, the main effect will be retrieved for the arithmetic mean of the baseline values using the centered variable for PaO_2/FiO_2. Multiple imputation will be applied in the intention to treat population of patients receiving at least one dose of treatment or the control.

Analysis of Secondary Variables

Statistical analysis of the prespecified secondary endpoints will be performed with descriptive and exploratory statistical methods according to the scale and observed distribution (absolute and percentage frequencies, chi-square tests, logistic regression models for categorical variables; means and standard deviations, medians and quartiles, or ranges with t-tests or Mann-Whitney tests and linear regression models for continuous variables; Kaplan-Meier curves, log-rank tests and Cox proportional hazard models for censored data). P-values will be reported but should not be considered part of the confirmatory analysis.

Subgroup Analysis

Planned subgroup analyses will be performed according to the following:

- Sex and race (only for subgroups larger than 40 subjects)
- Patients with increased pulmonary arterial pressure
- Direct vs. indirect lung injury

- Age stratified by decades

Stopping Rules

For safety reasons, after the enrolment of 20 patients (day 28 after last dose IMP Patient 20), an interim analysis of the following will be performed:

1. An increased risk of pulmonary hemorrhage ≥°III according to CTCAE Version 5.0 in the treatment (iloprost) arm and
2. Levels of IMP in the serum.

The results will be discussed with the DSMB. The DSMB has to assess whether the result allows continuation of the study as planned.

Moreover, after treatment of a total of 100 patients (day 28 after the last dose IMP Patient 100), an interim analysis of an increased risk of pulmonary hemorrhage ≥°III according to CTCAE Version 5.0 in the treatment (iloprost) arm will be performed, and the results will be discussed with the DSMB. The DSMB has to assess whether the result allows continuation of the study as planned.

Moreover, in the case of the following situations, a premature termination of the trial must be considered:

1. Substantial changes in risk-benefit considerations
2. New insights from other trials and
3. Insufficient recruitment rate.

Biometric Report

The biometric report will be delivered according to the SOP BI07 of the statistical center (IKEaB). In summary, the report will contain sections on the statistical methodology, preprocessing of data, and the descriptive, exploratory, and confirmatory analyses. It will be reviewed by the PI before presenting the final version.

Discussion

To date, there is no pharmacologic intervention to treat or prevent the development of lung injury or ARDS. Iloprost-containing medications are well recognized epidemiologically as an effective therapeutic agent for the treatment of moderate to severe pulmonary hypertension. Iloprost has been shown to exert anti-platelet and anti-inflammatory actions in small clinical observation studies and several preclinical laboratory examinations. However, the use of iloprost for the treatment of ARDS is not novel and has been used in small studies before. Indeed, we propose in this study to systemically evaluate the application of iloprost in an RCT to identify the potential use and benefit of iloprost in ARDS. The composite endpoint was chosen as it is likely to be more sensitive than just 28-day mortality to detect an

effect signal. Although it is not a double-blinded strategy, the recorded objectives will help support or refute our hypothesis that iloprost reduces lung inflammation during early ARDS.

This study includes some possible pitfalls like the single blinded design. However due to randomization and based on the close data acquisition we will be able to minimize bias.

However, next to the effect of iloprost on lung inflammation, this study will also be a resource for information about clotting issues in terms of systemic and local anticoagulation effects of iloprost in lung tissue but also other compartments than lung. Although iloprost is used frequently in pulmonary hypertension, still there are no data about iloprost concentration in blood after inhalative treatment. In addition, iloprost may have a positive effects of lung compliance during acute ARDS as well as during during resolution, since it has been shown to have a lasting positive effect on fibrosis in lung and other tissues in animal models (34, 35). In one lung ventilation iloprost seem to reduce intrapulmonary shunts resulting in better oxygenation (36, 37). In this context the analysis of ventilator free days or time on ECMO may reveal important information. Further on, intravenous application of iloprost may improve microcirculation resulting in better kidney recovery in septic patients (37, 38). Patients suffering ARDS frequently show multiorgan failure. Therefore the comparison of incidence and time frame for extracorporeal therapy may give insights in the effect of inhaled iloprost on microcirculation in other organs. Therefore iloprost may influence positively the outcome of ARDS patients by at least one the effects described above. This study will be the first describing effects of iloprost on inflammation, fibrosis, bleeding events and oxygenation organ failure and anticoagulation during a continuous time frame of at least 5 days in critical ill patients.

Trial status

Patient recruitment started 5th of July 2019 based on the 4th version of the protocol released 22th May 2019 and approved by the ethics committee of the University Tuebingen, Germany (899/2018AMG1) at 4th of June 2019. The recruitment will be finished approximately in July 2021.

List Of Abbreviations

ADR Adverse drug reaction

AE Adverse event

AKIN Acute kidney injury

ALI Acute lung injury

ALT Alanine aminotransferase

ARDS Acute respiratory distress syndrome

AST Aspartate aminotransferase

BfArM Bundesinstitut für Arzneimittel und Medizinprodukte

BNP Brain natriuretic peptide

CAM-ICU Confusion assessment method for the ICU

CRRT Continuous renal replacement therapy

CD11b Fibrinogen receptor activation

CHE Cholinesterase

COX-2 Cycolooxygenase 2

CRF Case report form

CTCAE Common Toxicity Criteria for Adverse Events

CT Computed tomography

CVVHD Continuous veno-venous hemodialysis

CVVHDF Continuous veno-venous hemodiafiltration

CVVHF Continuous veno-venous hemofiltration

DAMP Damage-associated molecular pattern

DFG Deutsche Forschungsgemeinschaft

eCRF Electronic case report form

FiO_2 Inspiratory oxygen concentration

GCP Good clinical practice

HFO High-frequency oscillation

IC Informed consent

ICAM-1 Intercellular adhesion molecule 1

ICH International Conference on Harmonization of Technical Requirements for

Registration of Pharmaceuticals for Human Use

ICU Intensive care unit

IFN-γ Interferon gamma

IL-6 Interleukin 6

IMC Intermediate care

IMP Investigational medicinal product

INR International normalized ratio

IR Ischemia-reperfusion injury

ISF Investigator site file

ITT Intention to treat

MAP Mean arterial pressure

NET Neutrophil extracellular traps

NO Nitric oxide

P-selectin Platelet activation factor selectin P

PAH Pulmonary arterial hypertension

PAMP Pathogen-associated molecular pattern

PaO_2 Partial pressure of oxygen

PTT Partial thromboplastin time

PEEP Positive end expiratory pressure

PNC Platelet-neutrophil complexes

PPSB Prothrombin complex concentrate

PRR Pattern recognition receptor

RCT Randomized controlled trial

RAP-1 Ras-related protein 1

RASS Richmond Agitation Sedation Score

RR Blood pressure

SAE Serious adverse event

SLED Sustained low-efficiency dialysis

SOFA Sequential Organ Failure Assessment score

qSOFA Quick SOFA

SpO_2 Oxygen saturation

svO_2 Central venous oxygen saturation

TNF-α Tumor necrosis factor alpha

VASP-P Phosphorylation of vasodilator phosphoprotein

VCAM-1 Vascular adhesion molecule 1

VES Vulnerable Elderly Survey

vWF vonWillebrand factor

ZVD Central venous pressure

Declarations

Funding

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

PR and HAH drafted the current manuscript. MG, AZ, MK, PM and SP critically reviewed and revised the draft report. All authors have read and approved the final version, which was also approved by the sponsor.

Ethics approval and consent to participate

The Thilo Trial protocol was approved by the ethics committee of the University Tuebingen, Germany (Protocol Number: 899/2018AMG1) at 4th of June 2019. The Local Ethics Committee at each site will approve the study protocol (approvals already in place shown in Additional file 1). Any modifications to the protocol will be immediately communicated to all responsible authorities. All patients, or their legal representative, must give written informed consent.

Consent for publication

Not Applicable.

Competing Interests

The authors declare no competing interests.

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Tables

Table 1: Definition of ARDS

Mild ARDS	Moderate ARDS	Severe ARDS
200 mmHg < PaO_2/FiO_2 ≤ 300 mmHg PEEP ≥ 5 cm H_2O	100 mmHg < PaO_2/FiO_2 ≤ 200 mmHg PEEP ≥ 5 cm H_2O	PaO_2/FiO_2 ≤ 100 mmHg PEEP ≥ 5 cm H_2O

Table 2: Table of Events

Event	Presentation until start of Iloprost or NaCl 0.9%	d 1	d 2	d 3	d 4	d 5	d 6-27	Hospital Discharge or d28	Hospital Discharge or d90	d 180 ± 14d
Informed consent	X									
Inclusion/ Exclusion criteria	X									
Pregnancy test in women of childbearing age	X									
Demographics	X									
Medical History	X									
Randomization	X									
Iloprost or NaCl 0.9% (control)		X	X	X	X	X				
Clinical assessment including outcome	X	X	X	X	X	X	X	X	X	
Laboratory testing	X	X	X	X	X	X	X	X		
AE/ SAE monitoring		X	X	X	X	X	X	X		
Plasma biomarkers	X	X	X	X	X	X				
Barthel Index	X							X	X	X
SOFA score	X	X	X	X	X	X	X	X		
Health-related questionnaire										X

VES										X

Table 3: Study Assessments

		Iloprost administration								
Laboratory testing	**Admission**	**d 1**	**d 2**	**d 3**	**d 4**	**d 5**	**ICU/ IMC**	**Ward**	**Hospital Discharge or d28**	**Hospital Discharge or d90**
Blood count	X_1	X_1	X_1	X_1	X_1	X_1	X_2	X_3	X	X
Procalcitonin	X_1	X_1	X_1	X_1	X_1	X_1	X_2	X_3	X	X
IL-6	X_1	X_1	X_1	X_1	X_1	X_1	X_2	X_3	X	X
PaO_2/FiO_2	X_1	X_1	X_1	X_1	X_1	X_1	X_2	X_3		
Hemoglobin	X_1	X_1	X_1	X_1	X_1	X_1	X_2	X_3	X	X
Hemostasis parameters	X1	X1	X1	X1	X1	X1	X_2	X_3	X	X
Renal parameters	X_1	X_1	X_1	X_1	X_1	X_1	X_2	X_3	X	X
Clinical Parameters										
Ventilation support including ventilation parameters	X_1	X_1	X_1	X_1	X_1	X_1	X_1			
Prone positioning	X_1	X_1	X_1	X_1	X_1	X_1	X_1			
ECMO	X_1	X_1	X_1	X_1	X_1	X_1	X_1			
Relaxation	X_1	X_1	X_1	X_1	X_1	X_1	X_1			
High-frequency ventilation	X_1	X_1	X_1	X_1	X_1	X_1	X_1			
Tracheotomy										
Hemodynamic parameters	X_1	X_1	X_1	X_1	X_1	X_1	X_2	X	X	X
Vasopressor therapy	X_1	X_1	X_1	X_1	X_1	X_1	X_1			
Inotrope therapy	X_1	X_1	X_1	X_1	X_1	X_1	X_1			
Fluid balance	X_1	X_1	X_1	X_1	X_1	X_1	X_1			
Transfusion of red blood cells	X	X	X	X	X	X	X_2	X	X	X
Transfusion of thrombocytes	X	X	X	X	X	X	X_2	X	X	X
Anticoagulation										
Infection	X	X	X	X	X	X	X_2	X	X	X
Anti-infective therapy	X	X	X	X	X	X	X_2	X	X	X
Length of stay in ICU										
Length of stay in hospital										
Discharge location									X	X
Death										
Cause of death										
Scores										
Richmond Agitation Sedation Score (RASS)	X_1	X_1	X_1	X_1	X_1	X_1	X_1			
SOFA $score_4$	X	X	X	X	X	X	X		X	X
Barthel Index									X	X

1: Assessment on daily basis during ICU stay

2: Assessment on daily basis until day 14 and then once per week during ICU/IMC stay

3: Assessment once per week on ward

4: SOFA score during ventilation support once per week

5: qSOFA Score spontaneous breathing

Figures

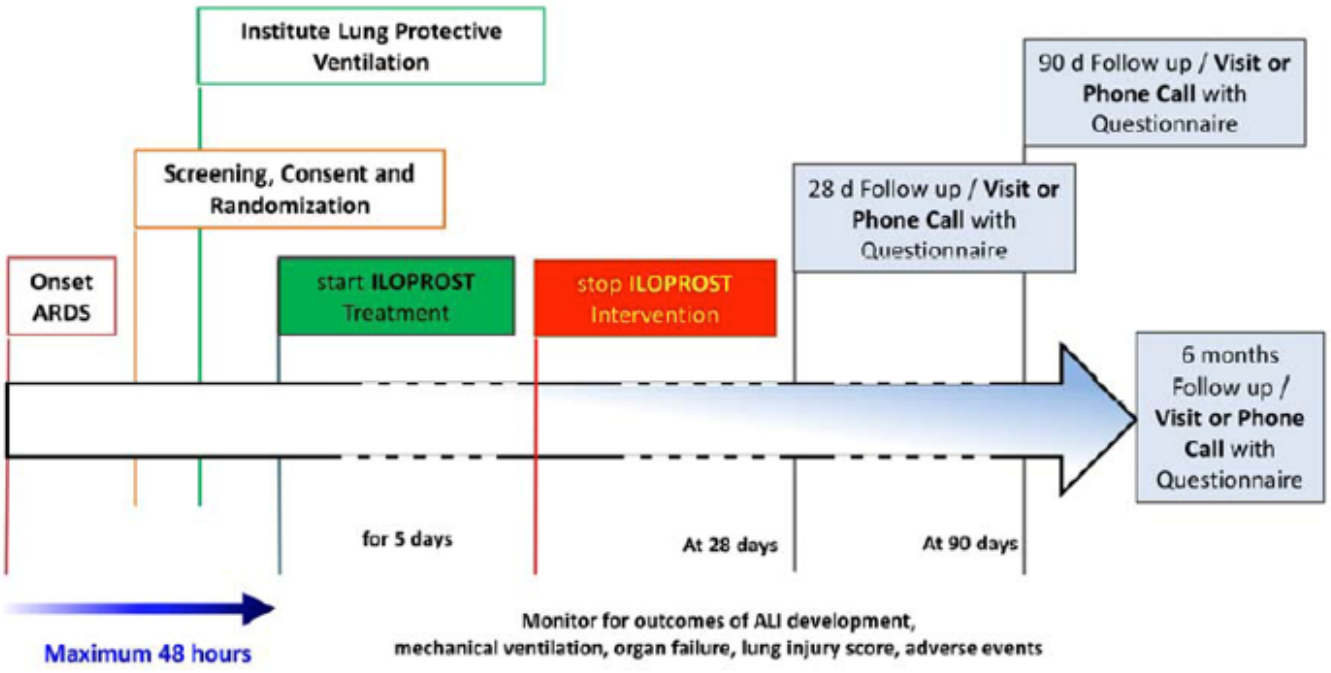

Figure 1

Trial protocol and intervention scheme. After screening and determination of eligibility, patients will be included after a maximum of 48 hours after the onset of ARDS. Within this time period, screening, consent and randomization will be initialized. In addition, lung protective ventilation will be instituted. After randomization, Iloprost 3x20 µg (intervention) or NaCl 0.9% (control) will be administered for 5 days through a standard ultrasound nebulizer. Daily recordings will be made with respect to the development of the PaO2/FiO2 ratio and the severity of ARDS, organ failure, lung injury and potential adverse events. The treatment with Iloprost or NaCl (0.9%) will be stopped after 5 days. The follow-up period will then

continue up to 90 days and 6 months to determine the outcome, quality of life and pulmonary/secondary organ function.

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