S t u d y P r o t o c o l

BIOMARKER-GUIDED IMPLEMENTATION OF ANGIOTENSIN II TO REDUCE THE OCCURRENCE OF KIDNEY DAMAGE AFTER CARDIAC SURGERY (ANGIOTENSIN II TO REDUCE KIDNEY DAMAGE)

ACRONYM

(AIDED-TRIAL)

EudraCT-No.: 2021-003088-87

Sponsor:
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Sponsor's study code:
WWU20_0016

Final V1.2
NCT05199493

TABLE OF CONTENTS

1 General Information

1.1 Synopsis

Title:	Biomarker-guided implementation of angiotensin-II (AT-II) to reduce the occurrence of kidney damage after cardiac surgery
Short Title:	Aided-Trial
Sponsor's Study Code:	WWU20_0016
EudraCT-No.:	2021-003088-87
Sponsor:	Westfälische Wilhelms-Universität Münster
Investigator:	Univ.-Prof. Dr. med. A. Zarbock
Indication:	Cardiac surgical patients at high risk for AKI
Study Design:	Single-centre clinical Trial Two-arm, randomised, blinded, controlled, parallel-group trial
Investigational Medicinal Product:	Test product: Angiotensin II acetat (Giapreza 2,5 mg/ml) Reference therapy: Placebo (0.9 % NaCl)
Therapy	After identifying cardiac surgery patients at high risk for AKI by renin levels (Δ-renin (difference between post- and pre-operation) ≥ 3.7 µU/ml 4h after CPB) who require vasopressors, patients will be randomly allocated to the control or intervention group. Experimental intervention: Angiotensin II will be added to the first-line vasopressor (norepinephrine) and the angiotensin II dose and norepinephrine will be adjusted so that the mean arterial pressure is above 65 mmHg and the first-line vasopressor (norepinephrine) can completely be weaned.. Control intervention: The control substance (0.9% NaCl) will be added to the first-line vasopressor (norepinephrine) and the dose of the control substance and norepinephrine will be adjusted so that the mean arterial pressure is above 65 mmHg. The treatment with study medication will be performed for 12 hours. If the patient requires a vasopressor after the intervention period, the first-line vasopressor (norepinephrine) is administered..
Objectives:	The primary objective of this trial is to investigate the efficacy of AT-II vs. standard of care on the biomarkers [TIMP-2]*[IGFBP7] in high-risk patients undergoing cardiac surgery. HYPOTHESIS: Superiority of AT-II in cardiac surgery patient with a hyperreninemia and vasopressor use compared to standard of care with respect to kidney damage (personalized approach).
Endpoints:	Primary Endpoint: • kidney damage after cardiac surgery identified by the difference between [TIMP-2]*[IGFBP7] levels 12h after

	randomization and [TIMP-2]*[IGFBP7] levels at randomization Secondary Endpoints: • occurrence of AKI within 72h after cardiac surgery (according to the KDIGO criteria) • occurrence of moderate and severe AKI within 72h after cardiac surgery (according to the KDIGO stage 2 and 3) • amount of volume application • transient (<72h) and persistent (<72h) AKI • fluid status • dose and duration of vasopressor use • creatinine clearance on day one after cardiac surgery • free-days through day 28 of vasoactive medications and mechanical ventilation • renal recovery at day 90 (renal recovery is defined as serum creatinine levels < 0.5 mg/dl higher than baseline serum creatinine (creatinine level before surgery) • 30-day, 60-day and 90-day mortality • length of ICU stay • length of hospital stay • use and duration of renal replacement therapy within hospital stay • use of RRT at days 30, 60, 90 • $MAKE_{90}$ (major adverse kidney events consisting of mortality, dialysis dependency, persistent renal dysfunction (defined as serum creatinine ≥ 2x compared to baseline value) at day 90 • Effect of ACEi/ARBs use on the AT II effect • Correlation between the severity of hyperreninemia and the AT II effect Other variables: • Surveillance of vital parameters on ICU • Safety laboratory parameters • Occurrence of thromboembolism • Adverse events Add-on study: • determination of further biomarkers (e.g. KIM-1, chitinase, NGAL, hyaluronic acid) and anti- and pro-inflammatory mediators (IL-6, IL-10…)
Planned Number of Study Centers:	1
Number of Patients/ Sample size:	Expected [TIMP-2]*[IGFBP7] level in the control group: median=0.84, Q1=0.35, Q3=1.57 and in the AT-II group: median=0.58, Q1=0.26, Q3=1.20 (based on Meersch et al., Intensive Care Med 2017). A total number of 64 patients will be recruited and a number of 32 patients will be randomized to each of the two treatment arms. With an expected 5% dropout rate, up to 2 patients per treatment arm will be non-evaluable. With a number of n=30 evaluable patients per treatment arm, the location shift between the distributions of the biomarker [TIMP-2]*[IGFBP7] in the AT-II and the control group can be

	estimated with an expected precision ±0.35ng/ml^2/1000 (half-width of the 95% confidence interval).
Inclusion Criteria:	**Inclusion criteria for registration** 1. Adult patients scheduled for cardiac surgery with cardiopulmonary bypass (CPB), 2. 18 years of age or older, 3. cardiac index > 2.1 l/min per square meter, 4. Written informed consent. **Inclusion criteria for randomization** Registered patients will be randomized only if 1. Δ-renin (difference between post- and pre-operation) ≥ 3.7 µU/ml 4h after CPB. 2. postoperative hypotension requiring vasopressors
Exclusion Criteria:	1. Preexisting AKI (stage 1 and higher) 2. Patients with cardiac assist devices (ECMO, LVAD, RVAD, IABP) 3. Pregnant women, breastfeeding women and women of childbearing potential 4. Known (Glomerulo-) Nephritis, interstitial nephritis or vasculitis 5. Chronic kidney disease with eGFR < 20 ml/min/1.73m^2 6. Dialysis dependent CKD 7. Prior kidney transplant within the last 12 months 8. Emergency surgery in the context of an acute coronary syndrome 9. Hypersensitivity to the active substance, or to any of the excipients of the study medication 10. Bronchospasm 11. Liver failure 12. Mesenteric ischemia 13. Participation in another intervention trial in the past 3 months 14. Persons with any kind of dependency on the investigator or employed by the institution responsible or investigator 15. Persons held in an institution by legal or official order
Visits:	Screening: T1: Screening T2: Surgery until 4h after terminating CPB Treatment: T3: Operation Day Observation: T4: POD 1 T5: POD 2 T6: POD 3 T7: POD 7/Discharge T8: Follow-up POD 30 T9: Follow-up POD 60 T10: Follow-up POD 90
Statistical Methods	<u>Efficacy:</u> The randomized groups will be descriptively compared on all baseline variables using summary statistics such as mean and standard deviation, median and quartiles, or frequency and percentage, as appropriate. In inferential statistical analyses two-sided significance tests will be applied with a local

	significance level alpha=0.05. All point estimates of parameters of interest will be supplemented by 95% confidence intervals unless otherwise stated. SAS or SPSS statistical software will be used for all data analyses. Description of the primary efficacy / test accuracy analysis and population: The primary efficacy analysis will include all randomized subjects (full analysis set) and will be performed according to the intention-to-treat principle, i.e. all subjects are analyzed in the group to which they were randomized. Additional sensitivity analyses will be performed according to the per-protocol principle. The effect of AT-II versus placebo on the biomarker [TIMP-2]*[IGFBP7] will be estimated with a non-parametric 95% confidence interval of the location shift between the distributions of the biomarker [TIMP-2]*[IGFBP7] in the AT-II and the placebo group, respectively. Safety: Safety data will be evaluated descriptively, including all recruited study patients who received at least one dose of study therapy (safety population). Results are reported by mean parameter estimates and associated 95% confidence intervals. Secondary endpoints: Statistical analysis of the pre-specified secondary endpoints will be performed with descriptive and inferential statistical methods.
Financial Support:	The project is supported by the DFG
Schedule	Planned start date of the study (FPFV): 01.10.2021 Planned end date of recruitment (LPFV): 15.04.2022 Planned end date of the study (LPLV): 15.07.2022 Planned recruitment duration: 6,5 months Duration of single patient participation: 90 days Planned overall duration of the study: 9,5 months

1.2 Flow Chart

	T1	T2		T3	T4-T6	T7	T8	T9	T10
Visit	**S[1]**	**R[2]**	**B[3]**	**OD**	**POD 1-3**	**Dis-charge /POD 7**	**POD 30**	**POD 60**	**POD 90**
Informed Consent	X								
Inclusion and Exclusion criteria	X								
Renin measurement before and after cardiac surgery (4 hours after cardiopulmonary bypass)			X	X					
Registration		X							
Demographic data Age, sex, BMI, comorbidities (EuroSCORE, hypertension, coronary artery disease, congestive heart failure, NYHA, peripheral vascular disease, diabetes, previous stroke or transient ischemic attack, chronic liver disease, chronic kidney disease, chronic obstructive pulmonary disease, cancer), medication (b-blockers, ACEi/ARBs, diuretics, anticoagulation)			X						
Admission diagnosis, Category of admission (elective, emergency)			X						
Surgical parameters nature of surgical procedure, duration of surgical procedure, duration of CPB, duration of aortic cross clamp				X					
Randomization				X					
Urinary [TIMP-2]*[IGFBP] measurement At randomization and 12 h after start of intervention				X					
Intervention / Study medication				X					
APACHE II				X					
Hemodynamics (MAP, HR, CVP)				X	X				
Pressors				X	X				
SOFA-Score				X	X				
KDIGO criteria					X				
Fluid balance / 24h urine volume				X	X				
Concomitant Medication N-acetylcysteine, amphotericin, aminoglycosides, cyclosporine, tacrolimus, radiocontrast agents, diuretics				X	X				
Safety laboratory test Complete blood count, potassium-, sodium-, , creatinine and BUN and eGFR, pH, bicarbonate, bilirubin, CRP, PCT, coagulation parameter			X		X				
Add-on study				X[4]	X[5]				
Mortality						X	X	X	X
Length of stay (ICU, Hospital)						X		X	
Duration of ventilator support / duration of administration of vasopressors						X	X		
Number of days of RRT/RRT dependence						X			X
(Serious) adverse events				X	X	X	X	X	X

[1] Screening

[2] Registration

[3] Baseline (prior operation until randomization)

[4] Blood and urine samples will be collected 4h after CPB, 12h and 24h after start of study medication

[5] Blood samples will be collected on POD 2 and 3

1.3 Abbreviations

ADL	Activities of Daily Living
AE	Adverse Event
AMG	Arzneimittelgesetz
AKI	Acute Kidney Injury
BfArM	Federal Institute for Drugs and Medical Devices (Bundesinstitut für Arzneimittel und Medizinprodukte)
CKD	Chronic kidney disease
CRRT	Continuous Renal Replacement Therapy
CRF	Case Report Form
CTCAE	Common Terminology Criteria for Adverse Events
DMC	Data Monitoring Committee
DSUR	Development Safety Updat Report
ICU	Intensive Care Unit
eCRF	Electronic Case Report Form
FPFV	First patient first visit
GCP	Good Clinical Practice
ICH	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
IMP	Investigational Medicinal Product
ISF	Investigator Site File
KDIGO	Kidney Disease: Improving Global Outcomes
ZKS	Zentrum für Klinische Studien
LPFV	Last patient first visit
LPLV	Last patient last visit
NA	Not applicable
ND	Not done
OD	Operative day
POD	postoperative day
RAAS	Renin-angiotensin-aldosterone system
RRT	Renal Replacement Therapy
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SAS	Statistical Analysis System
SOFA	Sequential Organ Failure Assessment
SOP	Standard Operating Procedure
SUSAR	Suspected Unexpected Serious Adverse Reaction
TMF	Trial Master File

2 Introduction

Vasoplegic syndrome is a form of distributive shock that is characterized by low arterial pressure with reduced systemic vascular resistance and normal or elevated cardiac output[1] that occurs in 5 to 25% of patients undergoing cardiac surgery. Patients with vasoplegia after cardiac surgery are at higher risk of organ failure, including acute kidney injury (AKI)[2, 3]. Postsurgical AKI is associated with several adverse outcomes. Attempts to prevent AKI have largely been futile so far. Prior studies often started with the interventions after an AKI event, when a decline of kidney function (i.e. glomerular filtration rate) was already established. Application of norepinephrine is currently considered as the first-line therapy for vasoplegia, but all catecholamines have adverse effects, including myocardial ischemia and arrhythmias[4]. In a recent observational trial, we demonstrated that there is a dysregulation in the renin-angiotensin-aldosterone system (RAAS) likely caused by a reduced angiotensin-converting enzyme (ACE) activity after cardiac surgery. Elevated renin levels identified patients at risk for AKI and were associated with cardiovascular instability and increased AKI rate after cardiac surgery[5]. Furthermore, elevated renin levels could be used to identify high-risk patients for cardiovascular instability and AKI who would benefit from timely intervention with angiotensin II (AT-II) that could improve their outcomes[5]. Therefore, the application of AT-II to treat a postoperative hypotension would mean a hormone substitution. We hypothesize that the application of AT-II added to standard of care in cardiac surgery patients with a high risk for AKI as identified by AKI biomarkers reduces kidney damage.

2.1 Background Information and Rationale

Acute kidney injury (AKI) can be defined by changes of serum creatinine and/or urine output, according to the KDIGO criteria[6]. In cardiac surgery patients, the AKI rate is up to 30%, with 1–2% of patients requiring renal replacement therapy (RRT)[7-9]. Cardiac-surgery associated AKI (CSA-AKI) is associated with increased short- and long-term morbidity and mortality as well as increasing hospital costs[7-9].

Shock after cardiac surgery is associated with increased mortality[10, 11]. Cardiopulmonary bypass (CPB) represents a common clinical setting of sympathetic nervous system activation and cardiovascular instability. Vasoplegia is a form of distributive shock that is characterized by low arterial pressure with reduced systemic vascular resistance and normal or elevated cardiac output[1]. It occurs in 5 to 25% of patients undergoing cardiac surgery. Patients with vasoplegia after cardiac surgery are at higher risk of organ failure, including AKI, and have an increased mortality rate and longer hospital length of stay[2, 3].

Application of norepinephrine is currently considered as the first-line therapy for vasoplegia but all catecholamines have adverse effects, including myocardial ischemia and arrhythmias[4]. In addition, in vasoplegic states, vascular smooth muscle cells may become unresponsive to catecholamines. The underlying mechanisms are complex and include adrenoceptor desensitization, increased nitric oxide synthesis, activation of adenosine triphosphate–sensitive K+ channels, and vasopressin and corticosteroid deficiency[12].

The renin-angiotensin-aldosterone system (RAAS) is a hormone system that plays a central role in regulating blood pressure and fluid, glomerular filtration rate, and electrolyte balance[13, 14]. Renin, a proteolytic enzyme released by juxtaglomerular cells in response to hypotension, decreases sodium delivery to the distal tubule, activates the sympathetic nerve system, and cleaves angiotensinogen to angiotensin (AT)-I which is a precursor of the vasoactive AT-II. The RAAS system is regulated by a biofeedback loop. AT-II generation inhibits renin release, whereas renin levels increase when there is insufficient activation of the AT-II type 1 receptor. Administration of Angiotensin Converting Enzyme (ACE) inhibitors and AT-II receptor blockers and reduced AT-II generation cause a corresponding increase in renin levels.[15, 16]

Despite numerous clinical trials using several interventions, [17] a reliable means to prevent AKI remains elusive. Clinical trials focusing on septic patients suggest that AT-II is a potent vasopressor. However, no human data exist whether the application of AT-II in high-risk

patients identified by renin levels (individualized approach) reduces kidney damage and improves kidney function after cardiac surgery. Furthermore, leukocyte function and mediators modulating inflammation will be investigated in an add-on study.

2.2 Risk-Benefit Analysis

Patients included in this trial are at high risk for AKI. Currently, there is no pharmacologic option available to prevent the development of AKI. However, it is well known that AKI is associated with an 8-fold increased risk for the development of chronic kidney disease (CKD) as well as end-stage renal disease and an increased risk for mortality.
AT-II is a synthetic peptide hormone which is approved for refractory hypotonia in adults in septic or other forms of distributive shock. Vasoplegia, the clinical condition treated in this clinical trial, is a form of distributive shock. Preliminary data have shown that treatment with AT-II reduced hyperreninemia in cardiac surgical patients with vasoplegia. As hyperreninemia is supposed to be associated with AKI [5], it is conceivable that a treatment of AT-II reduces kidney damage. Known interactions or side effects of AT-II are limited to thromboembolic complications. Therefore, patients with any form of prothrombotic conditions will not be included in this trial. Moreover, patients with hypersensitivity to the active substance, or to any of the excipients of the study medication will be excluded from the trial. The application of AT-II will be gradually decreased since patients may experience hypotension or worsening of the underlying diagnosis of shock on abrupt withdrawal or premature discontinuation if complications occur that might be related to the use of AT-II. The burdens and predictable risks will be closely supervised by the treating physicians. To mitigate the risk of thromboembolic events in the clinical trial laboratory tests of coagulation (e.g. PTT, INR, Thrombocytes and – if applicable antifactor Xa are implemented to detect and treat hypercoagulation during the treatment period (see 8.4.3 Rescue Medication and Emergency Measures). Furthermore – depending on the operation and the previous illnesses – all patients are started with at least prophylactic anticoagulation on the first evening.

Patients assigned to the control group receive only standard care and may not have a treatment benefit. However, since a renoprotective effect is still unknown, patients participating in the trial have the same risks as not participating in the trial. Patients with vasoplegia receive vasopressors in order to increase the systemic vascular resistance (SVR) and subsequently the mean arterial blood pressure (MAP). This ensures organ perfusion and oxygen delivery. Norepinephrine is currently the first-line treatment to treat vasoplegia induced by different causes. However, other vasopressors are also available which can increase the SVR and MAP. Although the available vasopressors (norepinephrine, vasopressin, angiotensin II) have different mode of actions, they are all used to secure organ perfusion. As both groups (intervention group and control group) have the same MAP target, leaving out norepinephrine and replacing it by another vasopressor is without an increased risk for the patient.

This trial may result in a substantial improvement in the management of patients at risk of AKI with a potential improvement of patients' outcome. A participation in this trial will not influence the therapeutic treatment of patients after cardiac surgery. All patients receive standard treatment according to the current standards.

3 Objectives and Endpoints

3.1 Objectives

3.1.1 Primary Objective

The primary study outcome is kidney damage after cardiac surgery identified by measuring the biomarkers [TIMP-2]*[IGFBP7] in high risk.

3.1.2 Secondary Objectives

Secondary objectives include:

- Occurrence of AKI
  The rate of AKI within 72h after cardiac surgery will be evaluated according to the KDIGO guidelines using both serum-creatinine and/or urinary output criteria.

- Occurrence of moderate and severe AKI within 72h after cardiac surgery
  The rate of moderate and severe AKI within 72h after cardiac surgery will be evaluated according to the KDIGO stages 2 and 3 .

- Creatinine clearance on day one after cardiac surgery

- Free days through days 28 of vasoactive medications and mechanical ventilation

- Renal recovery at day 90
  Recovery of renal function will be defined as serum creatinine levels < 0.5 mg/dl higher than baseline (level prior to surgery).

- Mortality at day 30, 60, and 90

- Vasopressor use

- Fluid application and volume status

- ICU length-of-stay AND Hospital length-of-stay
  Both ICU and hospital length-of-stay will be defined based on the ICU and acute hospital admissions during patients' randomization. Length-of-stay will be evaluated on the basis of both the mean number of days of ICU/hospital stay following randomization and Kaplan- Meier survival, censored for patient drop out or death. Hospital discharge will be defined as discharge from acute care, whether to acute rehabilitation, transitional care, long-term care or home.

- Use and duration of renal replacement therapy within 72h after cardiac surgery, and 30, 60 and 90 days after randomization
  The duration of renal support will be defined as the number of days from the initiation of RRT to final dialysis treatment (last data collection 90 days after randomization). Duration of renal support will be censored if the patient is still dialysis dependent at the time of death. Duration of renal support will be evaluated on the basis of both the mean number of days of renal support and Kaplan-Meier survival, censored for patient death. The optimal outcome in AKI is the ability of the patient to return to his or her prior living situation not requiring RRT on an ongoing basis.

- Major adverse kidney events at day 90
  Major adverse kidney event (MAKE) will be defined as the composite of death, use of renal replacement therapy and persistent renal dysfunction (defined as serum

creatinine ≥ 2x compared to baseline value) at day 90.

3.1.3 Additional Research Program

To understand the mechanisms of illness and recovery and how AT-II affects them, further biomarkers will be measured (e.g. KIM-1, NGAL, renin, HA and anti- and proinflammatory mediators (IL-6, IL-10…)). Blood and urine samples from recruited patients will be collected 4h after CPB, 12h, and 24h after start of intervention. The details of the additional research program will be contained in a separate plan, which will be finalized before the final analysis.

3.2 Endpoints

3.2.1 Primary Endpoint

The primary study endpoint is

- **Difference between [TIMP-2]*[IGFBP7] levels 12h after randomization and [TIMP-2]*[IGFBP7] levels at randomization.**

3.2.2 Secondary Endpoints

- occurrence of AKI within 72h after cardiac surgery (according to the KDIGO criteria)
- occurrence of moderate and severe AKI within 72h after cardiac surgery (according to the KDIGO stage 2 and 3)
- amount of volume application within 12h after randomization
- fluid status 12h after randomization
- dose of vasopressor use during intervention
- creatinine clearance on day one after cardiac surgery
- free-days through day 28 of vasoactive medications and mechanical ventilation
- renal recovery at day 90 (renal recovery is defined as serum creatinine levels < 0.5 mg/dl higher than baseline serum creatinine (creatinine level before surgery)
- 30-day, 60-day and 90-day mortality
- length of ICU stay
- length of hospital stay
- use and duration of renal replacement therapy within hospital stay
- use of RRT at days 30, 60, 90
- $MAKE_{90}$ (major adverse kidney events consisting of mortality, dialysis dependency, persistent renal dysfunction (defined as serum creatinine ≥ 2x compared to baseline value) at day 90
- Effect of ACEi/ARBs use on the AT II effect
- Correlation between the severity of hyperreninemia and the AT II effect

Other variables:

- Surveillance of vital parameters on ICU
- Safety laboratory parameters
- Occurrence of thromboembolism
- Adverse events

Add-on study:

- determination of further biomarkers (e.g. KIM-1, chitinase, NGAL, renin, hyaluronic acid and anti- and pro-inflammatory cytokine levels (IL-6, IL-10…), leukocyte functions

4 Study Design

The Aided-trial is a single center, prospective, interventional, phase IIIa, two-arm parallel group, randomized-controlled, double blinded trial including 64 patients at high risk for the development of AKI after cardiac surgery with CPB.

Discussion of trial design
To the best of our knowledge, no trial has been performed investigating the effect of AT-II on kidney damage in patients at high risk for AKI undergoing cardiac surgery. Our preliminary data demonstrate that elevated renin levels were associated with cardiovascular instability and increased rates of AKI after cardiac surgery. In order to investigate whether AT-II can also prevent kidney damage in high-risk patients undergoing cardiac surgery, we will perform a feasibility trial. If we can demonstrate that AT-II mitigates kidney damage in high-risk patients undergoing cardiac surgery, a follow-up trial could investigate whether AT-II can significantly reduce the occurrence of AKI after cardiac surgery. Therefore, this study is necessary to demonstrate that biomarker-guided implementation of AT-II can mitigate kidney damage after cardiac surgery. If the trail is positive, we will use the data and study protocol to apply for an interventional trial showing that renin-level-guided AT-II application reduces kidney damage after cardiac surgery.

5 Study Sites and Study Population

5.1 Study Site Selection

The study will be performed as a single-center randomized-controlled trial. The Department of Anesthesiology, Intensive Care and Pain Medicine at the University Hospital of Muenster has a lot of experience with randomized-controlled trials. The study team consists of experienced investigators and study nurses. The measurement of [TIMP-2]*IGFBP7] will be performed with the NephroCheck® test (Astute, San Diego, CA, USA) which is available at the corresponding cardiac surgical ICU. The IMP will be prepared and the applied through independent personnel to ensure blinding of the process.

5.2 Study Population

Patients at high risk for AKI after cardiac surgery with CPB will be identified by hyperreninemia (∆-renin (difference between post- and pre-operation) ≥ 3.7 µU/ml 4 h after CPB).

5.2.1 Inclusion Criteria

Inclusion criteria for registration
1. Adult patients undergoing cardiac surgery with CPB
2. 18 years of age or older
3. Cardiac index > 2.1 l/min per square meter
4. Written informed consent

Inclusion criteria for randomization
Registered patients will be randomized only if
1. ∆-renin (difference between post- and pre-operation) ≥ 3.7 µU/ml 4 h after CPB
2. Postoperative hypotension requiring vasopressors

5.2.2 Exclusion Criteria

1. Preexisting AKI (stage 1 and higher)

2. Patients with cardiac assist devices (ECMO, LVAD, RVAD, IABP)
3. Pregnant women, nursing women and women of childbearing potential
4. Known (Glomerulo-) Nephritis, interstitial nephritis or vasculitis
5. CKD with eGFR < 30 ml/min
6. Dialysis dependent CKD
7. Prior kidney transplant within the last 12 months
8. Emergency surgery in the context of an acute coronary syndrome
9. Hypersensitivity to the active substance, or to any of the excipients of the study medication
10. Bronchospasm
11. Liver failure
12. Mesenteric ischemia
13. Participation in another intervention trial in the past 3 months
14. Persons with any kind of dependency on the investigator or employed by the institution responsible or investigator
15. Persons held in an institution by legal or official order

5.2.3 Distribution of Gender in the Study Population

We expect a gender distribution of (male: female) 70:30. No patient will be excluded from the study on the basis of gender. Gender will be used for covariate adjustment in the final analysis. A subgroup analysis will be performed according to gender.

6 Patient Inclusion, Registration and Randomization

Patients who are cared for at the study sites and who are possibly eligible will be invited for study participation until the desired number of patients is achieved. Once written informed consent for participation has been given (cf. section 15.215.2), a subject identification code will be assigned.

After successful registration, when patients meet all designated inclusion criteria (including identifying a high risk patient (Δ-renin (difference between post- and pre-operation) ≥ 3.7 µU/ml 4 h after CPB), and none of the exclusion criteria, patients will be randomized using an internet-based randomization tool at https://www.randomizer.at. Randomization will be performed in a proportion of 1:1.
The time interval between patient fulfilling eligibility criteria and start of infusion may not exceed 30 minutes.

7 Study Medication

7.1 Investigational Medicinal Product:

The trial sites will use the study medication as article of trade, which will be used routinely in the hospital.
Patients randomized to the intervention group will receive AT-II (10,000ng/ml Giapreza®) for 12 h after randomization. The starting dose will be 20 ng/kg/min and the dose will be adjusted (up to 80 ng/kg/min) so that the other vasopressors can completely weaned and the mean arterial pressure is > 65 mmHg. Patients randomized to the control group will receive placebo (0.9 % NaCl).

7.1.1 Drug Characterization

7.1.1.1 Name of the medicinal product

Giapreza®, concentrate for solution for infusion

Control/Placebo: Isotone Natriumchloridlösung 0,9 % Braun Infusionslösung.

7.1.1.2 Qualitative and quantitative composition

Giapreza®,
1 ml contains:
Active substance Quantity
Angiotensin-II-Acetat 2.5mg
osmolarity: 130 to 170 mOsmol/kg
pH value: 5.0 - 6.0

Control/Placebo:
100 ml contains: Natrium chloride: 0,9 g
Electrolyte concentrations: Na^+: 154 mmol/l, Cl^-: 154 mmol/l
Theoretical osmolarity: 308 mosmol/l
Titration acidity (pH 7,4): < 0,3mmol/l

7.1.1.3 List of excipients

Giapreza®:
Excipients: Mannitol, Water for injections, Sodium hydroxide (for pH adjustment), Hydrochloric acid (for pH adjustments

Control/Placebo:
Water for injections

7.1.1.4 Pharmacodynamic properties

Giapreza®
Angiotensin II (AT II)increases blood pressure by vasoconstriction; the increased release of aldosterone by direct action of AT-II on the vessel wall is mediated by binding to the G-protein-coupled AT-II receptor type 1 on vascular smooth muscle cells, stimulating Ca2+/calmodulin-dependent phosphorylation of myosin and causing smooth muscle contraction.
GIAPREZA is titrated for each individual patient by effect. In the ATHOS-3 study, the median time to increase in blood pressure was approximately 5 minutes. The effect on blood pressure is sustained for at least the first three hours of continuous intravenous infusion. Because of the short half-life of GIAPREZA (less than one minute), abrupt discontinuation of AT may result in rebound hypotension.
Therefore, after sufficient improvement of the underlying shock, slow down-titration in increments of up to 15 ng/kg per minute, as controlled by blood pressure values, is recommended as needed.

Control/Placebo:
Sodium is the major cation of the extracellular fluid space and regulates its size along with various anions. Sodium and potassium are the main carriers of bioelectrical processes in the organism.
The sodium content and fluid metabolism of the organism are closely linked. Any change in plasma sodium concentration that deviates from the physiological level simultaneously influences the fluid status of the organism. Regardless of the serum osmolality, an increased sodium content means an increased fluid content or a reduced sodium content of the organism a decrease in body water.
A 0.9% sodium chloride solution corresponds to plasma osmolarity. Upon delivery of this solution, it comes first to replenish the interstitium, which makes up about 2/3 of the

extracellular space. Only about 1/3 of the volume delivered remains intravasal. The solution is thus hemodynamically effective only for a short time.

7.1.1.5 Pharmacokinetic properties

Giapreza®
No specific studies have been conducted to investigate the metabolism and excretion of GIAPREZA. The half-life of intravenously administered AT-II in plasma is less than one minute. It is metabolized by end cleavage (at both the amino and carboxy terminals) in various tissues, including erythrocytes, plasma, and major organs (e.g., intestines, kidneys, liver, and lungs).
No trials have been conducted to investigate the pharmacoginetics of AT-II in renally impaired patients and/or in patients with hepatic impairment since the kidneys and the liver are not a major organ for AT-II metabolism or excretion.

7.1.1.6 Shelf-life

Giapreza®
3 years.
To be used immediately after the bottle is opened.
Giapreza® is not to be stored after addition of other components.

Control/Placebo:
3 years.
To be used immediately after the bottle is opened.

For microbiological reasons, the ready-to-use preparations should be used immediately. Normally, blends with other components should not be stored for more than 24 hours at 2°C – 8°C unless they have been prepared under controlled and validated aseptic conditions

7.1.1.7 Special precautions for storage

Giapreza®
Store in a refrigerator (2-8°C). For storage conditions after dilution of the medicinal product see 7.1.1.6.

Control/Placebo:
This medicinal product does not require special storage conditions.

7.1.1.8 Marketing authorization holder

Giapreza®: PAION AG, Aachen, Deutschland

Control/Placebo: B. Braun Melsungen AG, Melsungen, Deutschland

7.1.1.9 Marketing authorization numbers

Giapreza®: EU/1/19/1384/001; EU/1/19/1384/002; EU/1/19/1384/003

Control/Placebo (Isotone Natriumchloridlösung 0,9% Braun): 6697366.00.00; 6726174.00.00

7.1.2 Contraindications

Giapreza®: Hypersensitivity to the active substance or to any of th excipients listed in 7.1.1.2

Control/Placebo:

None

7.1.3 Special Warnings and precautions for use

Giapreza®
The clinical experience with GIAPREZA is limited to septic or other distributive shock. The use of GIAPREZA is not recommended in other types of shock (e.g. cardiogenic shock, etc aspatients with non-distributive shocks were excluded from clinical trials.
Thromboembolic events have reported with the use of angiotensin II in clinical trials. The major imbalance compared to placebo was in venous thromboembolism (6.1% vs 0%). Concurrent venous thromboembolism prophylaxis should be used unless contraindicated during treatment with GIAPREZA. Non-pharmacologic venous thromboembolism prophylaxis may be considered where pharmacologic prophylaxis is contraindicated.
Peripheral ischaemia has been reported with the use of AT-II. It is important to administer GIAPREZA at the lowest compatible dose to achieve or maintain adequate mean arterial pressure and tissue perfusion.

Control/Placebo:
Isotonic sodium chloride solution 0,9% should be used with caution in
- Hypernatremia
- hyperchloremia

7.1.4 Interaction with other Medicinal Products and other Forms of Interaction

Giapreza®:
No interaction studies have been performed. No invitro metabolism studies have been performed with GIAPREZA.
Concomitant administration of GIAPREZA and other vasopressors may have an additive effect on mean arterial pressure (MAP). The addition of GIAPREZA may require a reduction in doses of other vasopressors.
Patients who have recently received angiotensin converting enzyme (ACE) inhibitors may be more sensitive to GIAPREZA's action with an increased response. Patients who have recently received angiotensin II receptor blockers (ARBs) may be less sensitive to GIAPREZA's actions with a reduced response

Control/ Placebo:
No interactions are known to date.

7.1.5 Undesirable Effects

Giapreza®: The most frequent adverse reactions (≥1/10) reported more often are thromboembolic events (12.9% vs 5.1%) and transient hypertension. Common adverse reaction ((≥1/100 to < 1/10) were Tachycardia and peripheral ischaemia.)

Control/Placebo: Side effects are not expected under normal use.

7.1.6 Administration

After identifying cardiac surgery patients at high risk for AKI by renin levels, patients will be randomly allocated to the control or intervention group.

Giapreza®
Solution for infusion after mixture with a compatible infusion solution should be infused via the

central venous route.

Daily dose
Giapreza will be added to the first-line vasopressor (norepinephrine) and the dose will be adjusted so that the mean arterial pressure is above 65 mm Hg and first-line vasopressor can be weaned to a low level. The recommended starting dosage of GIAPREZA is 20 nanograms (ng)/kg per minute via continuous intravenous infusion.
GIAPREZA must be diluted in sodium chloride 9 mg/ml (0.9%) solution for injection prior to use. One or two millilitres of GIAPREZA must be diluted in sodium chloride 9 mg/ml (0.9%) solution for injection to achieve a final concentration of 5,000 ng/ml or 10,000 ng/ml.

When initiating GIAPREZA, it is important to closely monitor blood pressure response and adjust dose accordingly.
Once an infusion has been established, the dose may be titrated as frequently as every 5 minutes in steps of up to 15 ng/kg per minute, as needed, depending on the patient's condition and target mean arterial pressure. Approximately one in every four patients experienced transient hypertension with the angiotensin II 20 ng/kg per minute starting dose in clinical trials (see 7.1.5), thus needing dose down-titration. For critically ill patients, the usual target mean arterial pressure is 65 – 75 mmHg. Do not exceed 80 ng/kg per minute during the first 3 hours of treatment. Maintenance doses should not exceed 40 ng/kg per minute. Doses as low as 1.25 ng/kg per minute may be used.
It is important to administer GIAPREZA at the lowest compatible dose to achieve or maintain adequate arterial blood pressure and tissue perfusion (see 7.1.3). The median duration of treatment in clinical trials was 48 hours (range: 3.5 to 168 hours).
In order to minimise the risk of adverse events derived from prolonged vasoconstriction, treatment with GIAPREZA should be withdrawn once underlying shock is sufficiently improved (see 7.1.3 and 7.1.5). Down-titrate by gradual decrements of up to 15 ng/kg per minute, as needed, based on blood pressure, in order to avoid hypotension due to abrupt withdrawal (see 7.1.3).

Control/Placebo
The Placebo will be added to the first-line vasopressor (norepinephrine) and the dose of the control substance will be adjusted so that the mean arterial pressure is above 65 mm Hg.

7.1.7 Dose Selection Rationale

Patients allocated to the intervention group will receive Giapreza® according to the package leaflet for a time of 12h.

7.1.8 Dose Modification

In case of the occurrence of expected or unexpected side effects, GIAPREZA should be gradually decreased since patients may experience hypotension or worsening of the underlying diagnosis of shock on abrupt withdrawal or premature discontinuation.

7.1.9 Overdose

Overdose may result in severe hypertension. Down-titration of therapy, careful observation, and initiation of appropriate supportive measures are the indicated treatment of overdose of angiotensin II. Hypertensive effects are expected to be brief because the half-life of angiotensin II is less than one minute.

7.1.10 Manufacturing, Blinding and Labeling

The trial site will use the study medications as article of trade, which will be used routinely in the hospital. Labeling and blinding will be performed by medical staff who are independent from the AIDED investigational team. Fluids and matching placebo will be packaged in identical drug syringes. Each package will contain 250 ml of fluids and will be labeled with a subject identification number.

7.1.11 Supply, Drug Accountability, Storage Conditions, Disposal

Giapreza® from PAION AG will be used. The study site is responsible for ordering GIAPREZA at least 7 working days before it is needed. At the study site the receipt of Giapreza® including date of receipt, amount, batch number and expiry date will be documented. The investigator is responsible for the correct storage at the study site in a locked room. GIAPREZA must be stored at 2 to 8°C.

For each dispensed GIAPREZA or Control/Placebo to a patient, the subject identification code, the date of release, the batch number, and the expiry date will be recorded on the drug accountability form.

8 Treatment Plan

8.1 Treatment Plan

Renin will be measured 4h after CPB. Patients with a ∆-renin (difference between post- and pre-operation) ≥ 3.7 µU/ml 4 h after CPB are at high risk and will be randomized. Immediately after randomization (not longer than 30 min after fulfilling eligibility criteria), patients will receive intravenous infusions with the investigational drug or the control drug, according to 7.1.6.

8.2 Dose Modification/ Termination of Treatment

For dose modification see 7.1.8.
When the treatment is terminated, the nature of the termination must be documented (scheduled end or premature termination/discontinuation). In the event of premature termination/discontinuation, justification has to be given and it is to be recorded who took the decision to discontinue.

8.3 Control of Patients Compliance

It is not necessary to control/monitor the patient compliance. The administration of the investigational medicinal product is carried out exclusively by the investigator and/or the attending physician and is controlled by these persons and documented in the medical record.

8.4 Concomitant Medication / Therapy

The concomitant medication is not restricted.

8.4.1 Permitted Concomitant Medication / Therapy

Any medication / therapy for the treatment of the underlying disease is allowed.

8.4.2 Forbidden Concomitant Medication / Therapy

There is no forbidden concomitant medication / therapy.

8.4.3 Rescue Medication and Emergency Measures

Thromboembolic events are very common side effects of Angiotensin II in distributive shock. To mitigate the risk of thromboembolic events laboratory tests of coagulations are implemented at regular time points.

Patients with normal risk profile will receive prophylactic heparine (unfractionated heparin or low-molecular weight heparin.
The target apTT will be 40-60 s (unfractionated heparin).
The target antifactor Xa will be 0.1-0.39 IU/ml.

Patients with higher risk profiles and/or therapeutic heparin have the following thresholds:
Target aPTT: 60-100 s
Target antifactor Xa: 0.4 – 0.90 IU/ml

8.5 Pregnancy, Breast Feeding and Fathering of a Child

As there is currently insufficient data on administration of GIAPREZA to pregnant women, nursing mothers and children, administration of GIAPREZA is not recommended.

This study does not include pregnant women. For women of childbearing potential, a pregnancy test must be performed before inclusion in the clinical trial. Inclusion in the clinical trial is not possible if the test result is positive.

Breastfeeding women are also not included in the study.

8.6 Continuation of Treatment after the End of the Clinical Trial

Specific post-treatment is not required. The study interventions do not have consequences for the subsequent medical care. According to the current state of knowledge, there is no indication for the occurrence of long-term consequences after a therapy with Giapreza®. Nevertheless, a follow-up at day 30 and 90 will be conducted to clarify the objectives of the clinical trial.
The further treatment is carried out according to the local standard of the study site.

8.7 Unblinding

Emergency unblinding can be done through the randomizer through dedicated authorized personnel (Investigator / Safety Desk). To unblind a randomization, first display the list of randomizations (using the "Randomizations" link from the menu), then, in the row corresponding to the randomization to unblind, click on the "Unblind" link. Enter a reason why you want to break the randomization's blinding, confirm the operation by entering your password and click on the "Unblind" button. The randomization details, including the treatment name, will be displayed and users subscribed to unblind notifications will be notified.

Unless the trial user has the access right to unblind randomizations of all sites, only randomizations made by the user's site can be unblinded.

In case the Randomizer is not available the trial coordinator saved a local copy of the treatment codes (blinding tables) for emergency purposes.

Unblinding may only occur when this is relevant for the safety of the patient. Any premature code break (e.g. unblinding due to a Serious Adverse Event, accidental unblinding) should promptly be documented and reported to the sponsor including explanation/justification.

9 Study Conduct and Study Assessments

9.1 Screening (and Baseline) Visit

All patients scheduled for cardiac surgery with CPB will be documented in a screening log. If a patient fulfills all inclusion criteria and does not have any exclusion criteria ("eligible"), it should be documented in the screening log whether inclusion in the clinical trial has occurred. If the patient is not included in the clinical trial, the cause should be documented in the screening log.
A patient must provide written consent before undergoing any protocol-required assessments.

T1 Screening (Day -1)
- Verification of in-/exclusion criteria, including informed consent

Registration
Registration of patient

T1 Baseline (after registration)
- Demographic data, admission diagnosis, category of admission
- Safety laboratory test
- Concomitant medication

9.2 Intermediate Visits

T2 Baseline (during surgery until 4 h after terminating CPB)
- Nature of surgical procedure
- Time of skin incision, time of skin suture
- Duration of CPB
- Duration of aortic cross clamp
- Fluid input and output
- Hemodynamics (MAP, HR, CVP)
- Concomitant Medication / Pressors

T3 End of surgery until the 1st post-operative morning
- APACHE II
- SOFA-Score
- Hemodynamics
- Concomitant medication / Pressors
- Urinary [TIMP-2]*[IGFBP] measurement (at randomization and 12 h after start of intervention)
- Urinary renin test
- Blood and urine samples for laboratory evaluations (4 h after CPB, 0, 12, and 24 h after randomization)

- Randomization (after obtaining 4h renin test result)
- Start of study medication
- IMP-application
- Fluid input and output
- KDIGO-criteria
- Adverse events / reactions
- Transfer to ICU/intermediate care

T4 – T6 1st, 2nd and 3rd post-operative morning

- Safety laboratory test
- SOFA-Score
- KDIGO-criteria
- Hemodynamics (MAP, HR, CVP)
- Fluid input and fluid output
- Concomitant Medication / Pressors
- Plasma samples for laboratory evaluation (POD 2 and POD 3) (Add-on-Trial)
- Adverse events / reactions

9.3 Study Termination Visit

7 4th until 7th post-operative morning, or hospital discharge, whatever occurs first

- Need of RRT
- Length of stay (ICU, Hospital)
- Duration of ventilator support
- Major adverse kidney events
- Adverse events / reactions

9.4 Follow up

T8 (day 30 after surgery ±5 days)

- KDIGO criteria
- Duration of ventilator support
- Mortality

T9 (day 60 after surgery ±10 days)

- Mortality
- Need of RRT
- Length of hospital stay/ICU stay (if not already documented at T6)

T10 (day 90 after surgery ±14 days)

- Mortality
- Need of RRT
- Major adverse kidney events

9.5 Study Assessments

- Blood and urine samples for Add-o-Trial

Blood and urine samples will be collected at 4h after CPB and at 0, 12, and 24h after randomization through routinely placed catheters. The samples will be centrifuged and stored immediately at -80°C until measurement of biomarkers (e.g. NGAL, KIM-1). New renal biomarkers will be assessed. For this the samples will be stored not more than 10 years.

- Safety laboratory test

- complete blood count (thrombocytes included),
- potassium-, sodium-, ionized and total calcium levels
- creatinine, creatinine-clearance, BUN and eGFR,
- pH, bicarbonate
- bilirubine,
- CRP
- PCT
- Coagulation parameter (INR, prothrombin time, thrombin time, fibrinogen, antithrombin)

- Acute kidney injury
  - AKI will be defined according to the KDIGO criteria (see 18.1.2)

- Length of hospital stay
  - Cumulative days from hospital admission until hospital discharge

- Length of ICU stay
  - Cumulative days from ICU admission until ICU discharge

- Major adverse kidney events (MAKE)
  - combined endpoint consisting of mortality, dialysis dependency, persistent renal dysfunction (defined as serum creatinine ≥ 2x compared to baseline value) at day 90

10 Assessment of Efficacy and Safety

10.1 Assessment of Efficacy

Kidney damage after cardiac surgery identified by the difference between [TIMP-2]*[IGFBP7] levels 12h after start of study medication and [TIMP-2]*[IGFBP7] levels at randomization

To understand the mechanisms of illness and recovery and how AT-II affects them, markers of inflammation, oxidative stress, cellular hypoxia, and coagulation will be measured. Furthermore, leukocyte functions will be investigated.

10.2 Assessment of Safety

10.2.1 Definitions

Adverse Events are defined according to the Directive 2001/20/EC, the European Detailed Guidance CT 3, corresponding to the relevant German definitions in the GCP-Verordnung.

Adverse Event (AE)

An adverse event is any untoward medical occurrence in a patient or clinical trial subject administered an investigational medicinal product and which does not necessarily have a causal relationship with this treatment.

Thus, any new unfavourable and unintended sign, symptom or disease, or clinically significant increase in the intensity of an existing sign, symptom or disease, should be considered as an AE, whether or not considered related to the investigational medicinal product.

A pathological finding, improved or unchanged in comparison to its status before first administration of the study drug, does not constitute an adverse event.

Adverse Reaction (AR)

An adverse reaction is any untoward and unintended response to an investigational medicinal product related to any dose administered.

All adverse events judged by either the reporting investigator or the sponsor as having a reasonable causal relationship to a medicinal product qualify as adverse reactions. The expression reasonable causal relationship means that there is evidence or argument to suggest a causal relationship.

The definition covers also medication errors and uses outside what is foreseen in the protocol, including misuse and abuse of the product.

Serious Adverse Event (SAE)

A serious adverse event is any untoward medical occurrence or effect that at any dose

1. results in death
2. is life-threatening at the time of the event
3. requires inpatient hospitalisation or prolongation of existing inpatient hospitalisation
4. results in persistent or significant disability or incapacity,
5. is a congenital anomaly or birth defect.

Inpatient hospitalisation is defined as any stay in hospital on the part of a trial subject that includes at least one night (midnight to 06:00).

Hospitalisation without underlying adverse event is not an SAE, e.g. admission to hospital as an inpatient planned before the first administration of the investigational treatment, for a pre-existing condition that has not worsened. Such a situation must be documented in the proper manner in the trial subject's medical records and eCRF.

6. Medical judgment should be exercised in deciding whether an adverse event is serious in other situations. Important adverse events that are not immediately life-threatening or do not result in death or hospitalization but may jeopardize the subject or may require intervention to prevent one of the other outcomes listed in the definition above, may also be considered serious.

For reasons of drug safety, the occurrence of a pregnancy during the conduct of this trial is to be regarded as serious.

Unexpected Adverse Reaction

“Unexpected” means that the nature, severity or outcome of the adverse reaction is not consistent with the applicable product information for the investigational medicinal product.

- Expected ARs are listed in the reference document, passage 4.8, Undesirable effects. Reference document is the respective Summary of Product Characteristics for an investigational medicinal product, as submitted to the competent Authority.
- The term “severe” is often used to describe the intensity (severity) of a specific event. This is not the same as “serious,” which is based on patient/event outcome or action criteria.
- Reports which add significant information on the specificity, increase of occurrence, or severity of a known, already documented serious adverse reaction constitute unexpected events.
- An increase in the rate of occurrence of an expected AR, which is judged to be clinically important, is considered as unexpected.

- An expected adverse reaction with fatal outcome has to be considered as unexpected as long as the fatal outcome is not explicitly mentioned in the reference document.

Suspected Unexpected Serious Adverse Reaction (SUSAR)

A suspected unexpected serious adverse reaction (SUSAR) is a serious adverse reaction that has been judged to be unexpected.

10.2.2 Period of Observation, Documentation and Assessment of Adverse Events

AEs and SAEs will be recorded from the time the first dose of study drug is administered, up to and including follow-up-Visit d90 (T10)

All AEs, including SAEs, have to be recorded in the trial subject's medical records and on the adverse event pages in the CRF, regardless of whether or not the AE is considered related to the use of study drug.

Documentation and assessment by the investigator includes:

- Diagnosis or Description of AE
  - If possible, a diagnosis rather than a list of signs, symptoms and laboratory abnormalities should be given.
- Date of onset and date of end of AE
- Seriousness (yes/no)
- Severity (graded according to the general Common Terminology Criteria for Adverse Events (CTCAE) v5.0 scale. A semi-colon indicates 'or' within the description of the grade.):
  - Grade 1: Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
  - Grade 2: Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL (preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.).
  - Grade 3: Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self care ADL (bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden).
  - Grade 4: Life-threatening consequences; urgent intervention indicated.
  - Grade 5: Death related to AE.
- Causality (reasonable possibility / no reasonable possibility)
  - Reasonable possibility: there are facts or arguments to suggest a causal relationship
  - No reasonable possibility: time relationship is improbable, and/or another explanation is more likely (e.g. disease or other drugs provide plausible explanation)
- Action taken with investigational medicinal product (Dose not changed, Dose reduced, Dose increased, Drug withdrawn, Unknown, Not applicable)
- Outcome (recovered/resolved, recovering/resolving, not recovered/not resolved, recovered/resolved with sequelae, fatal, unknown)

All AEs and SAEs must be followed up until the condition resolves or stabilizes. The investigator should ensure that adequate medical care is provided to a subject for any adverse events. Transferring a patient from ICU to a normal ward ensures medical care.

10.2.3 Reporting of Serious Adverse Events (SAE) by the Investigator

The investigator has to report any SAE immediately (“immediately”, without undue delay) after obtaining knowledge by fax or by encrypted email (if available) on the SAE form to the Safety Desk, Muenster, Germany. This applies regardless of severity (CTCAE v5.0) or whether or not the SAE is considered related to the use of study drug. Personal data have to be replaced by the trial patient number before forwarding any information.

Safety Desk Contact
Zentrum für Klinische Studien (ZKS) Münster
Von-Esmarch-Straße 62
48129 Münster
Germany
Phone: +49 (0)251 83 57109
Fax: +49 (0)251 83 57112
E-Mail: mssd@ukmuenster.de

Where possible, a diagnosis rather than a list of symptoms should be given. The investigator is responsible for assessment of seriousness, severity and causality of the SAE. The SAE form should be completed with as much information as possible. The investigator should not wait for full details before making the initial report.

Minimum information to be included in any initial report:

1. Unique patient number
2. SAE details
3. Details about administration of investigational medicinal product
4. Causality assessment of SAE to investigational medicinal product
5. Reporting investigator

The investigator must fax any relevant follow-up information as soon as possible. In case of death a copy of an autopsy protocol should be provided, if any. The investigator should answer any query on SAE reports as soon as possible.

In case the competent authority or an ethics committee would request details concerning a fatal case, the investigator has to supply the requested information.

In case the investigator gets knowledge of an SAE occurring after the end of the reporting period, for which he suspects a reasonable causal relationship to the investigational treatment, the investigator should also report such an SAE to the Safety Desk. Late SAEs do not have to be documented in the eCRF.

10.2.4 Assessment of Serious Adverse Events by the Sponsor

The Safety Desk will document each SAE, check it and query additionally required information. The Coordinating Investigator, or a named delegate, will review each SAE again for seriousness and relatedness.

The Coordinating Investigator or his delegate will also assess whether an SAR is expected or unexpected (SUSAR) according to the applicable reference document (see 10.2.1), and whether any SAE might influence the benefit-risk-ratio or might require changes in the conduct of the trial.

10.2.5 Legal Reporting Requirements of the Sponsor

It is the duty of the Safety Desk to inform the competent authority, the ethics committee, and participating investigators about all suspected unexpected serious adverse reactions (SUSARs) in accordance with legal requirements (immediately, fatal or life threatening SUSAR

by the latest within 7 days, detailed follow-up information within an additional 8 days, if any; all other SUSARs by the latest within 15 days). SUSAR follow-up reports will be submitted, as appropriate.

The Safety Desk will observe SUSAR cross reporting obligations with other trials of the same sponsor investigating any of the same active substances, if any.

The Coordinating Investigator is responsible for the ongoing safety evaluation of the trial. The Safety Desk and the Coordinating Investigator will inform each other immediately about any relevant safety information coming to their knowledge. In case of safety relevant issues (besides SUSAR) which require expedited reporting, the Safety Desk will support the Coordinating Investigator in submitting an appropriate report in due time. This includes issues which might materially alter the current benefit-risk assessment of an investigational medicinal product or that would be sufficient to consider changes in the investigational medicinal products administration or in the overall conduct of the trial as well as urgent safety measures to protect the subjects against any immediate hazard.

Annual safety reports will be prepared and submitted in accordance with legal requirements (Development Safety Update Report, DSUR). The reports will be trial specific reports covering both investigational medicinal products of the trial. Data lock point for the report will be the day before the anniversary of the first authorization of the trial by the competent authority. The Coordinating Investigator is responsible for providing the updated benefit-risk assessment and passages requiring medical assessment. The Data Management is responsible for providing information on subject exposure. The Safety Desk is responsible for preparing the template, adding the other parts of the report, finalizing it and submitting it to the competent authority and the ethics committee within 60 days of the data lock point. On request by the competent authority or the ethics committee, additional reports will be prepared.

Details of all AEs will be reported to the competent authority on request.

10.2.6 Pregnancies

In order to identify and follow-up on outcome of pregnancy and on any congenital abnormalities, a positive pregnancy test is reportable on an SAE form within the following time frame: a) during active trial participation, b) thereafter while pregnancy probably was already present during active trial participation. The report should be made as soon as the investigator gains knowledge of the event. Follow-up of a pregnancy will be done using specific additional questionnaires. The Safety Desk will supply these trial-adapted forms, when required. However, onset of pregnancy during trial participation is very unlikely, due to the intensive care setting. Information will be collected as far as covered by informed consent (e.g., information about the child needs to be covered by consent of the patient's partner, too). When required, the Project Management will check and assure coverage by informed consent.

10.3 Data Monitoring Committee

The Data Monitoring Committee consist of three independent clinicians and one independent statistician. The board will monitor the trial on a regular basis (every 4 months) and will assess the safety of the trial. Procedures governing the convening and execution of the responsibilities of the DMC will be specified separately in a written DMC Charter.

11 Study duration, Premature Termination of the Study

11.1 Study Duration

Start of the trial: Date of randomization of first patient

Planned end of recruitment	6,5 months (LPFV, last patient first visit)
Last Patient Last Visit:	3 months (LPLV, last patient last visit)
Planned duration of single patient participation:	90 days

11.2 Definition of the End of the Study

The study will end at the last visit of the last patient.

11.3 Premature Study Termination

11.3.1 Premature Termination of Study Participation of a Single Patient

A patient is free to withdraw consent and discontinue participation in the study at any time, without prejudice to further treatment according to standard clinical practice. In this case the patient will be asked to permit the conduct of the study termination visit (cf. section 9.3).
In certain circumstances the investigator may exclude a patient from further study participation irrespective of the patient's will. Reasons may be:

- treatment failure
- insufficient compliance
- further participation might jeopardize the patient's health or well-being
- termination of the entire study.

If, after treatment initiation, a patient's participation is realized not to agree with the inclusion and exclusion criteria, the principal investigator will decide on further participation.
In case of premature termination, the reason will be recorded on the CRF.

11.3.2 Premature Termination of the Entire Study

The sponsor may terminate the entire study for relevant medical or administrative reasons. Reasons for termination will be documented in detail. If, in the view of an investigator, there are ethical issues or any other issues contradicting study continuation, the principle investigator must immediately be informed. Particular reasons for termination of the study may be:

- insufficient recruitment
- serious problems with the quality of the study data which cannot be solved
- discovery of an unexpected, serious or unacceptable risk to patients enrolled in the study
- new scientific evidence suggesting that study continuation is not justified.

12 Statistical Considerations

The randomized groups will be descriptively compared on all baseline variables using summary statistics such as mean and standard deviation, median and quartiles, or frequency and percent, as appropriate. In inferential statistical analyses two-sided significance tests will be applied with a local significance level alpha=0.05. All point estimates of parameters of

interest will be supplemented by 95% confidence intervals unless otherwise stated. SAS or SPSS statistical software will be used for all data analyses.

Description of the primary efficacy / test accuracy analysis and population

The primary efficacy analysis will include all randomized subjects (full analysis set) and will be performed according to the intent-to-treat principle, i.e. all subjects are analyzed in the group to which they were randomized. Additional sensitivity analyses will be performed according to the per-protocol principle. The effect of AT-II versus control on the biomarker [TIMP-2]*[IGFBP7] will be estimated with a non-parametric 90% confidence interval of the location shift between the distributions of the biomarker [TIMP-2]*[IGFBP7] in the AT-II and the control group, respectively.

Safety

Safety data will be evaluated descriptively, including all recruited study patients who received at least one dose of study therapy (safety population). Results will be reported by mean parameter estimates and associated 95% confidence intervals.

Secondary endpoints

Statistical analysis of the pre-specified secondary endpoints will be performed with descriptive and inferential statistical methods. Secondary outcomes will be evaluated and compared between the randomized treatment arms using the following methods. In case of normally or non-normally distributed metric outcomes, Student's t test or the Mann-Whitney U test will be applied, respectively. Categorical outcomes will be analyzed using Fisher's exact test (2x2 tables) or the Chi² test (>2x2 tables). Survival data will be analyzed using the Kaplan-Meier method and the log-rank test. Hazard ratios will be estimated based on Cox proportional hazards models after checking the proportional hazards assumption based on the Schoenfeld residuals.

Multivariable statistical analyses

Multivariable statistical analyses will be applied in order to identify independent prognostic factors of clinical outcomes. The identified prognostic factors will allow determining risk groups of patients with expected outcomes of different severity. Survival data will be analyzed using multivariable Cox proportional hazards models after checking the proportional hazards assumption based on the Schoenfeld residuals. Binary outcomes will be analyzed by multivariable logistic regression. Model building will be performed using stepwise variable selection based on Akaike's information criterion (AIC) in order to identify relevant and meaningful independent variables of a respective outcome. Models will be fitted and model parameters will be estimated using Maximum Likelihood methods. The final models will be validated using cross-validation techniques.

Sample size

The expected median [TIMP-2]*[IGFBP7] level in the control group is median control=0.84 (Q1=0.35, Q3=1.57) and in the AT-II group median AT-II=0.58 (Q1=0.26, Q3=1.20), based on Meersch et al., Intensive Care Med 2017. A total number of 64 patients will be recruited and a number of 32 patients will be randomized to each of the two treatment arms. With an expected 5% dropout rate, up to 2 patients per treatment arm will be non-evaluable. With a number of n=30 evaluable patients per treatment arm, the location shift between the distributions of the biomarker [TIMP-2]*[IGFBP7] in the AT-II and the control group can be estimated with an expected precision ±0.285ng/ml^2/1000 (half-width of the 90% confidence interval).

13 Documentation, Data Management, Archiving

13.1 Patient Identification List

All subject data will be collected in a pseudonymized form. Every trial subject can be identified by a unique subject identification code. A confidential subject identification list which links the patients' names with the subject identification code will be stored in the investigator site file.

13.2 Source Data / Source documents

Source data are, within the meaning of the ICH E6 Guideline, all information in original records and certified copies of original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data will be documented in various source documents (e.g. hospital records, doctor's report, subjects' diaries or evaluation checklists, x-rays) and then entered into the electronic Case Report Form (eCRF).

The following study specific data will be recorded directly in the eCRF and therefore will be considered as source data:

- KDIGO-criteria
- [TIMP-2]*[IGFBP7]

The following study specific data will only be recorded electronically in separate files and is not going to be entered in the eCRF

- Biomarkers for add-on study (e.g. KIM-1, chitinase, NGAL, hyaluronic acid) and anti- and pro-inflammatory mediators (IL-6, IL-10…), leukocyte function)

13.3 Recording of Data / Case Report Form (CRF)

Data will be recorded electronically using an EDC (Electronic Data Capture) system. Only persons authorized to enter data into the eCRF will have access to the EDC system. All users will be trained to use the EDC system and will comply with the instructions in the study-specific user manual. They will have continuous access to the data and reports of subjects at their own study site. The investigator is responsible for ensuring that the study data will be documented correctly, completely and in a timely manner. A study team physician takes on responsibility for the collected data by signing electronically. The electronic signature according to FDA 21 CFR Part 11 is the legally binding equivalent of the study team physician's handwritten signature.

13.4 Data Management

For data management, the validated data management system MACRO™ v4 (Elsevier Limited) will be used. All entered data will be stored on servers of the University Hospital Muenster. The servers are located in a secure data center and behind a firewall in the network of the University Hospital Muenster. A backup of the data will be saved on a daily basis and all data changes will be recorded in an audit trail.

All data will be checked for plausibility during initial data entry. Missing or non-plausible data are highlighted by the system right at input at the clinical study site and may be corrected immediately. Thereafter, according to the data validation plan, further data checks will be performed with regard to completeness and plausibility by the data management of the ZKS Muenster. In case of non-plausible or missing data, queries will be sent to the study site. The queries must be resolved by authorized members of the investigator`s staff in the respective study site in a timely manner.

After completion of data entry and data processing, the database will be locked and the data will be exported for statistical analysis. The investigator will receive a CD-ROM of the eCRF data for archiving at the clinical study site.

13.5 Archiving

After the end of the trial, the originals of all trial-specific documents (Trial Master File) including originals of the CRFs must be stored by the sponsor according to national regulations. Furthermore, the investigator stores the ISF (Investigator Site File) including copies of the CRFs for the time period given above.

No trial data or documents must be destroyed without prior written agreement between the sponsor and the investigator(s) or his/her designee.

13.6 Investigator Site File

The Investigator Site File (ISF) will be checked regularly for completeness and actuality during monitoring. After the clinical trial is finished or stopped, the ISF has to be stored according to national regulations.

14 Monitoring, Audits and Inspections

14.1 Monitoring

In order to ensure a high degree of data quality, the study site will be monitored during the recruitment and follow-up period (frequency depending on the site's recruitment).
The objectives of the monitoring procedures are to ensure that the study safety and rights of the study subjects as a study participant are respected and that accurate, valid and complete data are collected, and that the study is conducted in accordance with the study protocol, the principles of GCP and local legislation.
The principal investigator agrees that the monitor will visit the study site in appropriate intervals. During these visits the monitor will check the quality of the data recording and ensure that the study site adheres to the timeframe as set in the study protocol. The investigators agree to provide any relevant information and documentation whenever the monitor requires this information. This includes access to all original study documents and source data.

It is the responsibility of the investigator to keep the participant's chart as complete as possible (e.g. history, concomitant diseases, inclusion in the clinical study, visit dates, results of laboratory tests, distribution of the study medication, and adverse events). Source data are checked and compared with entries in the eCRF. The participant has given consent with this procedure by signing the patient information and written informed consent form. Additional tasks of the monitor are:

- to check, whether the study site fulfils requirements of the clinical study (e.g. participant population, technical equipment),
- instruction of the investigators and personnel for the clinical study,
- to check the ISF for completeness and actuality,
- documentation of the status of the participant,
- matching of original data,
- to check SAE reports according to regulations.

The monitor has the responsibility to treat all information confidentially and to safeguard the integrity and personal privacy of the study participants.

The exact extent of the monitoring procedures is described in a separate monitoring manual.

14.2 Audits und Inspections

For the purpose of ensuring compliance with the study protocol, the principles of GCP and local legislation, the sponsor may initiate an audit at any study site during the study and after completion. All study-related documentation must be made available to the designated auditor(s).

In addition, representatives of the regulatory authorities may choose to inspect the study site at any time prior to, during, or after completion of the clinical study. In this case, all pertinent study data should be made available as requested to the regulatory authority for verification, or inspection purposes. The investigator has to be available during these visits.

15 Ethical and Regulatory Requirements

15.1 Declaration of Helsinki and Legal Requirements

The study will be conducted in compliance with the declaration of Helsinki (current version, October 2013, Fortaleza), the requirements of the current German drug law ("Arzneimittelgesetz"), the current legal provisions regarding data protection, and the principles of Good Clinical Practice.

The present study will not be started before the ethics committee has given a favorable opinion and an approval by the responsible federal authority has been obtained.

In case of substantial amendments, a new application will be submitted to the ethics committee and/or the competent federal authority. Changes will not be implemented unless the competent ethics committee has given a favorable opinion and/or the competent authority has granted an approval.

Issues, which always require a favorable opinion of the ethics committee are for example:

- change of the investigator or his deputy,
- changes in any documents addressed to study participants or in any study information addressed to potential study participants.

According to German drug law §67, the investigator is required to notify the local surveillance authority about the beginning, the end and a premature termination of the study, about any substantial amendments and if the study is on hold. These notifications will be performed by a delegate of the sponsor (GCP-V, §12(3)).
Additionally, the investigator is required to notify the corresponding federal authority of the beginning of the study.

15.2 Patient Information and Informed Consent

Prior to inclusion into the study, the investigator informs each patient about the nature, significance, implications, and risks of the study as well as about the patient's right to withdraw from study participation at any time point without any resulting disadvantage. Further, the patients will be informed about insurance that covers risks originating from the study. According to the insurance conditions, they are advised to immediately inform the investigator if they were treated for an emergency and not to undergo any other medical treatment without prior consultation with the investigator.

Additionally, patients are handed out the patient information sheet and the informed consent form which are provided for this study. Patient consent in study participation must be given in writing. Before informed consent is requested, patients are left sufficient time for consideration. They are provided the opportunity for clarification of any study issues.

According to the German drug law ("Arzneimittelgesetz") §40(2a), patients are informed that their disease related data are stored in a pseudonymized way and used for scientific evaluations. For study participation they must agree in writing.

The informed consent form is dated and signed by the patient and by the investigator. The originally signed informed consent form is archived in the investigator site file. A copy of the signed informed consent form (or a second original) is handed over to the patient together with a copy of the patient information sheet and a copy of the general conditions of the patient insurance.

If the patient is unable to write, in exceptional cases, instead of the written consent required, oral consent in the presence of at least one witness, who was also included when the patient was being informed may be given. The witness may not be a member of the investigating team. The orally given consent has to be documented in writing, dated and signed by the witness.

In case of any study issue which requires a change of the patient information sheet, patients already included into the study must, if relevant to them, be informed about these issues orally and in writing and their written consent in further study participation must be obtained.

15.3 Patient Insurance

As required by German drug law, patients will be insured against risks originating from the study.

Insurance: HDI-Gerling Industrie Versicherung AG
Riethorst 2
30659 Hannover.

15.4 Financing

The study is financed by a research grant from the German Research Foundation (ZA 428/21-1)

15.5 Adherence to the Protocol

The investigator must adhere to the protocol as detailed in this document. Substantial changes to the protocol will require a written favorable opinion from the corresponding ethics committee and written approval by the corresponding authority prior to implementation. This does not apply when the modification is needed to eliminate an immediate hazard to patients. Any deviations from the protocol must be fully documented in the source documentation and recorded and explained in the CRF (if applicable).

15.6 Data Protection

This study will be performed in compliance with the applicable data protection laws. Study personnel will handle all patient data in a strictly confidential way.

To prevent the identification of a person to whom study data belongs, study data will be pseudonymized by means of the patient identification number (cf. sections 6, 13.1). If patient documents (e.g., examination results) are transferred to an institution outside the study site, copies will be used on which the patient's name and initials are obscured and the patient identification number is indicated.

16 Reporting and Publication Policy

16.1 Reporting

16.1.1 Interim Report

Not applicable.

16.1.2 Final Report

After completion of the biometric evaluation, the principal investigator prepares a study report. The report includes all trial results, irrespective of whether favorable or not. It is signed by him and the person who is responsible for the evaluation.

Within 12 months after the end of the study, the principal investigator submits a summary of the final report to the ethics committee and to the competent federal authority. The summary is prepared in the format provided for report synopses by the ICH Guideline E3 “Guidance on Structure and Content of Clinical Study Reports”. After review by the competent federal authority the report is published on the web site of DIMDI (German drug law, §42 b).

According to European Commission Guideline 2012/C 302/03 the study results should be posted within 12 months to the EU Database on Clinical Trials (EudraCT).

16.1.3 Safety Reports

The rules for the annual safety reports are specified in chapter 10.2.5 **(**Legal Reporting Requirements of the Sponsor**)**.

16.2 Publication Policy

Any publication will take account of the ‘International Committee of Medical Journal Editors’ (ICMJE). Any published data will observe data protection legislation covering the trial subject and investigators.

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18 Appendices

18.1 Measurement of [TIMP-2]*[IGFBP7]

[TIMP-2]*[IGFBP7] will be measured with the Astute140® Meter. It employs a sandwich immunoassay technique and converts the fluorescent signals from each of the two immunoassays (TIMP-2 and IGFBP7) contained within the Nephrocheck™ test cartridge into a single numerical risk result (AKIRisk). The result is calculated as the product of the measured concentrations of the two biomarkers, divided by 1000

AKIRisk = ([TIMP-2] * [IGFBP7]) / 1000 (units = $(ng/ml)^2/1000$)

18.2 KDIGO criteria

Table 2 | Staging of AKI

Stage	Serum creatinine	Urine output
1	1.5–1.9 times baseline OR ⩾0.3 mg/dl (⩾26.5 µmol/l) increase	<0.5 ml/kg/h for 6–12 hours
2	2.0–2.9 times baseline	<0.5 ml/kg/h for ⩾12 hours
3	3.0 times baseline OR Increase in serum creatinine to ⩾4.0 mg/dl (⩾353.6 µmol/l) OR Initiation of renal replacement therapy OR, In patients <18 years, decrease in eGFR to <35 ml/min per 1.73 m^2	<0.3 ml/kg/h for ⩾24 hours OR Anuria for ⩾12 hours