

Document Type:	Final Protocol
Document Date:	31 July 2025
Study Title:	Double-blind, randomized, placebo-controlled trial to evaluate the efficacy and safety of ilofotase alfa in patients at risk for renal damage following open heart surgery.
Protocol Reference Number:	AP-recAP-CSA-RD-02-01
NCT Number:	NCT06168799

CLINICAL TRIAL PROTOCOL

DOUBLE-BLIND, RANDOMIZED, PLACEBO-CONTROLLED TRIAL TO EVALUATE THE EFFICACY AND SAFETY OF ILOFOTASE ALFA IN PATIENTS AT RISK FOR RENAL DAMAGE FOLLOWING OPEN HEART SURGERY

ILOFOTASE ALFA FOR PREVENTION OF RENAL DAMAGE AFTER CARDIAC SURGERY

EUCT No.: 2023-505859-45

US IND No.: 117605

UTN: U1111-1295-0827

Protocol no.: AP-recAP-CSA-RD-02-01

Sponsor: AM-Pharma B.V.
Stadsplateau 7
3521 AZ Utrecht, The Netherlands

Sponsor's signatory:

[REDACTED]

Coordinating investigator: Prof Dr Peter Pickkers, MD, PhD
Radboud University Nijmegen Medical Centre
Geert Grooteplein Zuid 10
6525 GA Nijmegen, The Netherlands

Protocol version and date: Final 4.0, 31-Jul-2025

Previous versions and date: Final 3.0, 29-May-2024
Final 2.0, 13-Nov-2023
Final 1.0, 25-Jul-2023

This study protocol must be kept strictly confidential. Disclosure of the contents (in whole or part) to third parties is permissible only with written consent of AM-Pharma B.V.

Revision chronology

- Original version: Version 1.0, dated 25-Jul-2023
- Version 2.0, dated 13-Nov-2023

The following changes were included in Version 2.0:

- Sections 5.1 and 5.3: A rationale why coronary artery bypass grafting (CABG) with 3 or more distal anastomoses was chosen as inclusion criterion was added. The association between a cross-clamp time of 60 minutes and the risk of acute kidney injury was explained.
 - Section 10.2: The expected numbers of patients with a baseline estimated glomerular filtration rate (eGFR) >45 mL/min/m² and with CABG with 3 or more distal anastomoses were added.
 - Section 11.1.5: The paragraph on the maximum administered single dose in healthy volunteers was rephrased for clarity. Information was added on the timing of dosing and the impact on peak exposure in case of non-compliance with the protocol.
 - Section 17.7.1: It was clarified that the baseline eGFR and type of surgery will be included as covariates in the analysis of covariance of the primary efficacy endpoint to account for the stratification factors.
- Version 3.0, dated 29-May-2024

The following changes were included in Version 3.0:

- Title page and Section 2: The address of the Sponsor was updated.
- Sections 1 and 24: The estimated times for the trial end and recruitment period of the trial were updated.
- Sections 1, 6.1, 19.1, 19.5, 22.6, and 23: Legislative and regulatory text has been updated to be applicable for all involved countries.
- Sections 1, 7, 17.4, and 17.7.1: Clarified that the sCreat value taken pre-surgery (i.e., not more than 24 hours before surgery) is the baseline value.
- Sections 1 and 7: Clarification of the exploratory endpoint; changes in biomarkers of AKI will be compared to the pre-surgery baseline value.
- Sections 1, 8.1, 8.2.1, 10.2, 17.7.1, and 17.7.3: Clarified that randomization will be stratified by the baseline eGFR.

- Sections 1, 7, 8.1, and 9.2 (inclusion criterion 2.a): The surgery type “aortic valve plus aortic root and/or ascending aorta (excluding aortic arch)” will be included in the trial.
- Sections 1, 7, 8.1, and 9.2 (inclusion criterion 3): The wording of the inclusion criterion 3 was changed to “Screening eGFR ≥ 25 mL/min/1.73m² and ≤ 65 mL/min/1.73m² (calculated at Screening using sCreat which can be maximally 28 days old and the 2021 CKD-EPI formula [1] without race correction (eGFR = $142 \times \text{Min}(\text{Scr}/\kappa, 1)^\alpha \times \text{Max}(\text{Scr}/\kappa, 1)^{-1.200} \times 0.9938\text{Age}$ [$\times 1.012$ if female])).
- Sections 1, 9.1, 10.2, and 17.1: The sample size was increased from approximately 150 to up to 250 patients (up to 125 patients per treatment group) in response to the expected increased variability after the addition of multiple sites. The expected composition (number of patients) of the proposed cohort regarding the Baseline eGFR and CABG with 3 or more distal anastomoses was adjusted accordingly. Adapted sample size calculation/Rational for potential sample size increase was included in Section 17.1.
- Sections 1 and 9.2 (inclusion criterion 3): For clarification, the 2021 CKD-EPI formula without race correction was added.
- Patients may be rescreened 24 hours before surgery, if the patient does not fulfill the inclusion criterion 3 at the screening visit; Table 1 and Section 9.4 were adapted accordingly.
- Sections 1 and 9.2 (inclusion criterion 4) and Section 15.4: The follicle-stimulating hormone level has to be determined at Screening in post-menopausal women of age ≤ 60 years. The inclusion criterion 4 was adjusted accordingly.
- Sections 1 and 9.3 (exclusion criterion 18): Specified that implantation of a defibrillator or permanent pacemaker within one week prior to surgery is excluded.
- Section 1 (Table 1): An erroneous pharmacokinetic sampling time was corrected in the footer. For clarification, the display of assessment times of surgical parameters and complications was adjusted. Missing adverse event (AE) assessment at Screening was added for consistency.
- Sections 1 (Table 1) and 11.2: The documentation of concomitant treatment was clarified.
- Sections 1 (Table 1), 12.1 and 12.3: Clarification added that Screening, Baseline and surgery can be performed on the same day and the assessments do not need

to be repeated (i.e., check of in-/exclusion criteria, pregnancy test, prior treatment, adverse events). Accordingly, Screening is possible until Day 1.

- Sections 1 (Table 1), 12.1 and 12.3: The sCreat for Baseline can be the same as the sCreat value for eligibility and randomization as long as the latter has been taken within 24 hours prior to surgery.
- Sections 1 (Table 1) and 15.6: If a physical examination is performed within 72 hours prior to surgery as standard of care on admission to hospital, it does not need to be repeated at Baseline and the data will be used for baseline assessment.
- Sections 1 (Table 1) and 15.6: If height and weight measurement is performed within 72 hours prior to surgery as standard of care on admission to hospital, it does not need to be repeated at Baseline and the data will be used for baseline assessment.
- Sections 1 (Table 1), 12.7, and 18: Clarified that sampling for safety laboratory has to be done in case of treatment discontinuation after first administration of the investigational product (IP) and premature termination in the trial.
- Section 10.3: Sites can use their own syringes that meet specifications for IP administration.
- Section 12.6: Time points of follow-up visits are clarified.
- Section 13.1: Clarified that the 24-hour interval for sample collection of sCreat at Days 2, 3, 4, and 5 will start after start of surgery. The instructions for sample collection when Screening, Baseline and surgery take place on the same day were added.
- Section 13.5: For blood sampling of biomarkers, a time window of ± 1 hour (to the sample time for sCreat) was added.
- Section 15.1.1: Form for common surgical complications considered not related to the IP and not classified as AE was added for documentation.
- Section 17.3: Definition of the analysis sets was adapted.
- Section 17.7.3: Clarified that the change in severity of AKI (KDIGO stage) will be calculated from Day 1 post-surgery through Day 5.
- Section 17.9.2: The urinalysis erroneously listed under statistical methods of the safety analysis was deleted as it is not included in the clinical laboratory.
- Section 17.9.3 Physical examination: Section was deleted and the specifications on physical examination were added in Section 17.6.

- Sections 19.3 and 19.4: Investigator (or authorized designee) was added where applicable.
 - In the document, pre-operative was consistently renamed to pre-surgery, where applicable.
 - Additionally, editorial changes were made, which have no influence on the conduct of the clinical trial.
- Version 4.0, dated 31-Jul-2025

The following changes were included in Version 4.0:

- Sections 1 and 24: The estimated times for the trial end and recruitment period of the trial were updated.
- Sections 1, 7, 13.2, **Error! Reference source not found.**, 17.7.2, 17.7.2.1, 17.7.2.2, and 17.7.3: The effect of ilofotase alfa treatment on the clinical outcome of patients undergoing cardiac surgery, as measured by major adverse kidney events (MAKE) 60, will be analyzed as secondary efficacy endpoint and not exploratory as initially planned. The primary efficacy endpoint and the secondary efficacy endpoint will be tested in a hierarchical way. Accordingly, the secondary efficacy endpoint was included in the estimands for the analysis (Section 7). The statistical analysis of the endpoint was described (Section 17.7.2.1), and the secondary endpoints section (Section 17.7.2) was therefore divided in efficacy and safety. To reflect the endpoints, the MAKE 60 assessment (secondary efficacy endpoint) was placed before KDIGO AKI stage (exploratory endpoints) in the protocol Section 13.
- Sections 1, 7, and 17.3: The overall incidence of KDIGO AKI over the first 5 days after surgery was added as exploratory endpoint.
- Sections 7, 17.3, 17.4, and 17.7.1: Wording in statistical analysis and the intercurrent events (Section 7) in particular were adapted to the currently planned statistical analysis.
- Additionally, editorial changes were made.

The changes have no influence on the conduct of the clinical trial.

1 Summary and flow chart

Clinical trial code

AP-recAP-CSA-RD-02-01

Title of the clinical trial

Double-blind, randomized, placebo-controlled trial to evaluate the efficacy and safety of ilofotase alfa in patients at risk for renal damage following open heart surgery

Principal investigator(s) and trial center(s)

Multicenter clinical trial in Europe

Coordinating investigator: Prof Dr Peter Pickkers at Radboud University Nijmegen Medical Centre in Nijmegen, The Netherlands

Clinical phase: 2

Trial duration (planned)

Trial start (first patient first visit):	Q4 2023
Estimated trial end (last patient last visit):	Q4 2025
Estimated recruitment period:	21 months
Expected duration of patient participation:	about 3 months (including Screening)

Trial periods

Pre-treatment period (up to 30 days), treatment period (1 day) and follow-up period (60 days)

Objectives and endpoints

Objectives	Endpoints
Primary	
To assess the effect of ilofotase alfa on renal function	Ratio between the highest value post-surgery (post-surgery Day 1 and Days 2, 3, 4, and 5) and the pre-surgery baseline value for serum creatinine (sCreat)
Secondary	
Efficacy: To assess the effect of treatment with ilofotase alfa on the clinical outcome in patients undergoing cardiac surgery	Major adverse kidney events (MAKE) 60, defined as died up to and including Day 61, have received or are receiving new renal replacement therapy (RRT) up to and including Day 61, or have a decrease in estimated glomerular filtration rate (eGFR) $\geq 25\%$ on Day 61 compared to the pre-surgery baseline eGFR reference value

Objectives	Endpoints
Safety: To assess the safety and tolerability of ilofotase alfa in patients undergoing open chest heart surgery	Incidence rate of adverse events (AEs) and serious AEs (SAEs) through Day 28
Exploratory	
To investigate incidence, duration, and severity of acute kidney injury (AKI)	- The area under the curve (AUC) of AKI (kidney disease improving global outcomes [KDIGO] sCreat), defined as duration (in days) and severity of AKI; measured over the first 5 days after surgery - The overall incidence of KDIGO AKI over the first 5 days after surgery
To assess the effect on surgery related complications	Postoperative surgery related complications within 28 days of surgery, assessed according to the Society of Thoracic Surgeons (STS) definition
To assess the duration of critical care after the scheduled surgery	Length of stay in the intensive care unit (ICU) in days after the initial surgery
To investigate biomarkers of AKI (optional after the end of the trial)	Changes in biomarkers of AKI compared to Baseline
Pharmacokinetics	
To investigate the pharmacokinetic (PK) properties of ilofotase alfa	Population (Pop)PK

Trial assessments

Efficacy

- sCreat
- MAKE 60 (including death, new RRT and decrease in eGFR $\geq 25\%$) after surgery
- KDIGO AKI stage
- Length of ICU stay after surgery
- Biomarkers analyzed in blood and urine (optional after the end of the trial)

Pharmacokinetics

- PK parameters for ilofotase alfa

Other

- EuroScore II
- STS (surgery related complications)

Safety

- AEs
- Safety laboratory
- Anti-drug antibodies (ADAs)

Methodology

This is a Phase 2, multi-center, randomized, double-blind, placebo-controlled, 2-arm parallel group design trial in patients at risk for renal damage (RD) following open heart surgery. After screening and baseline assessments, eligible patients will be randomized 1:1 to either 2 doses of 128 mg ilofotase alfa, or 2 doses of placebo control. Randomization will be stratified by baseline eGFR (≤ 45 mL/min/m² or >45 mL/min/m²) and type of surgery (coronary artery bypass grafting [CABG] with 3 or more distal anastomoses or CABG combined with valve surgery or aortic valve plus aortic root and/or ascending aorta [excluding aortic arch]). The patient will be treated with the investigational product (IP; ilofotase alfa or placebo) at the same day before and after the surgery (Day 1). Follow-up clinic visits will be performed daily up to Day 5 for efficacy, safety, and PK assessments. A safety follow-up can be performed by telephone at Day 28 (± 4 days). At Day 61 (+10 days), an end of trial (EoT) visit will be done at site for efficacy, safety, and ADAs assessments.

Investigational products

Test product: Ilofotase alfa, containing 128 mg of the active ingredient ilofotase alfa in aqueous buffer at a pH of 7.0; clear, colorless to slightly yellow concentrate for preparing a solution for infusion supplied in glass vials.

Matching placebo, containing aqueous buffer at a pH of 7.0; clear, colorless to slightly yellow concentrate for preparing a solution for infusion supplied in glass vials that are identical to the ilofotase alfa vials in order to maintain the blind.

After dilution of the concentrate in 50 mL sterile sodium chloride 0.9% for injection (isotonic saline), the IP will be administered by intravenous (IV) infusion for 1 hour via a central catheter (preferred route) or a peripheral line, separately from any other concomitant medication via a dedicated lumen of the catheter or a separate peripheral line, using a dosing syringe or infusion bag.

Dose: The ilofotase alfa dose is a fixed dose of 128 mg per infusion. Two infusions will be given at Day 1. The IP has to be administrated pre-surgery (up to 6 hours before, started at least 30 minutes before first incision) and immediately post-surgery at Day 1 (at least within 4 hours after closing the chest) by 1-hour continuous IV infusion with at least 1 hour between completion of the first administration and the start of the second administration.

Number of patients (total and for each treatment) planned

Up to 250 patients are planned to be enrolled, up to 125 patients per treatment group.

Patient population

The target population consists of adult male and female patients scheduled for CABG with 3 or more distal anastomoses or CABG combined with valve surgery that are at risk of developing RD based upon pre-operative impaired kidney function (eGFR ≥ 25 and ≤ 65 mL/min/1.73m²).

Inclusion criteria

At Screening and Baseline the following inclusion criteria must be met:

1. Age ≥ 18 years
2. Planned for one of the following types of open chest cardiovascular surgery with the use of cardiopulmonary bypass pump (CPB):
 - a. 1) combined valve and CABG surgery
 - 2) aortic valve plus aortic root and/or ascending aorta (excluding aortic arch)
 - b. CABG with 3 or more distal anastomoses
3. Screening eGFR ≥ 25 mL/min/1.73m² and ≤ 65 mL/min/1.73m² (calculated at Screening using sCreat which can be maximally 28 days old and the 2021 CKD-EPI formula [1] without race correction ($\text{eGFR} = 142 \times \text{Min}(\text{Scr}/\kappa, 1)^\alpha \times \text{Max}(\text{Scr}/\kappa, 1)^{-1.200} \times 0.9938^{\text{Age}} [\times 1.012 \text{ if female}]$)¹
4. Female patients of childbearing potential agreeing to use one of the following effective contraception methods within IP treatment and 14 days thereafter:
 - a. combined (oestrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation (oral, intravaginal, transdermal)
 - b. progestogen-only hormonal contraception associated with inhibition of ovulation (oral, injectable, implantable)
 - c. intrauterine device
 - d. intrauterine hormone-releasing system
 - e. bilateral tubal occlusion
 - f. vasectomized partner (with medical assessment of the surgical success)
 - g. sexual abstinence

Abstinence is only accepted as true abstinence when this is in line with the preferred and usual lifestyle of the patient. Periodic abstinence (e.g., calendar, ovulation, symptothermal, post-ovulation methods and withdrawal) is not an acceptable method of contraception.

Post-menopausal females (i.e., >60 years of age or no menses for 12 consecutive months without an alternative medical cause with confirmatory follicle-stimulating hormone level of ≥ 40 mIU/mL) do not require contraception during the trial.

5. Male patients agreeing to refrain from donating sperm, use a male condom when having sexual intercourse and in case their partner is of childbearing potential they must agree

¹ Scr is serum creatinine (mg/dL), κ is 0.7 for females and 0.9 for males, α is -0.241 for females and -0.302 for males, Min indicates the minimum of Scr/ κ or 1, and Max indicates the maximum of Scr/ κ or 1.

to use adequate and effective contraception method (see inclusion criterion 4) within IP treatment and 14 days thereafter

Exclusion criteria

A patient who meets any of the following exclusion criteria at Screening and/or Baseline is excluded from participation in this trial:

1. Body weight ≤ 55 kg
2. Known or suspected glomerulonephritis (other than diabetic kidney disease) or other systemic vasculitis
3. Confirmed or treated endocarditis requiring antimicrobial or antiviral treatment within 30 days prior to surgery or other current active infection requiring antimicrobial or antiviral treatment within 14 days prior to surgery
4. Known chronic liver disorder with Child-Pugh C classification
5. Current planned (or scheduled) surgical intervention under conditions of circulatory arrest, including planned deep hypothermic circulatory arrest
6. Planned surgery for aortic dissection
7. Use of left ventricular assist device (LVAD), or intra-aortic balloon pump or other cardiac devices, within 7 days prior to surgery
8. Received any investigational drug within 30 days or 5 half-lives (whichever is longer) prior to Day 1 or planned or current participation in another interventional study until the EoT visit has been completed
9. Previous receipt of ilofotase alfa
10. Not willing and able to understand the information on the nature, the scope, and the relevance of the trial and to provide voluntary, written informed consent to participate in the trial before any trial related procedures
11. Pregnant or nursing women
12. Known or suspected hypersensitivity to ilofotase alfa or any components of the formulation used
13. Any previous organ transplantation
14. Congenital heart disease
15. Diagnosed with AKI (as defined by KDIGO criteria) within 3 months prior to surgery
16. History of RRT
17. Cardiogenic shock or hemodynamic instability which require inotropes or vasopressors or other mechanical devices such as intra-aortic balloon counter-pulsation (IABP) within 24 hours prior to surgery
18. A requirement for any of the following within one week prior to surgery: implantation of a defibrillator or permanent pacemaker, mechanical ventilation, IABP, LVAD, other forms of mechanical circulatory support

19. Required cardiopulmonary resuscitation within 14 days prior to cardiac surgery
20. Ongoing sepsis (as defined by SEPSIS-3, the Third International Consensus Definitions for Sepsis and Septic Shock) within the past 2 weeks or, in the opinion of the investigator, an untreated diagnosed clinically significant infection (viral or bacterial) prior to or at Screening and before randomization
21. Medical condition which requires active immunosuppressive treatment (daily steroid doses of ≤ 10 mg are allowed)
22. Employees or relatives of the sponsor or the investigator

Statistical methods

Statistical analyses will be performed by the sponsor or its designee. Further details of the statistical analysis (including shells for tables, figures, and listings, and any imputation of missing data) will be described in a statistical analysis plan, which will be finalized before data base lock.

The primary endpoint is the ratio of the highest observed sCreat value post-surgery (post-surgery Day 1 and Days 2, 3, 4, and 5) and pre-surgery sCreat baseline value. The primary analysis will use the ratio (maximum post-surgery/Baseline) to compare ilofotase alfa and placebo groups by means of an analysis of covariance with pre-surgery sCreat baseline value, eGFR stratification category, and type of surgery as covariates.

The comparison of ilofotase alfa and placebo group with respect to the secondary efficacy endpoint (MAKE 60) will be performed by means of a logistic regression analysis with the pre-surgery sCreat baseline value, eGFR stratification category, and type of surgery as covariates.

The primary and the secondary efficacy endpoint will be tested in hierarchical order.

An independent data monitoring committee (DMC) will continuously monitor the safety of the trial. The DMC will review and evaluate unblinded safety data including suspected unexpected serious adverse reaction reports submitted to the authorities on an ongoing basis, and listings of related SAEs on a monthly basis. The DMC will follow an agreed charter and will provide written communication to the sponsor and the trial steering committee on its recommendation on trial continuation or discontinuation based on safety data. More details of the safety data review, and the analyses will be provided in the DMC charter.

PK evaluation

A model based (compartmental) evaluation is foreseen. Data will be added to an existing PopPK model and fitted to evaluate at least clearance and volume of distribution in this population. The influence of relevant covariates might be explored as well as a comparison with previous observations captured in the PopPK model.

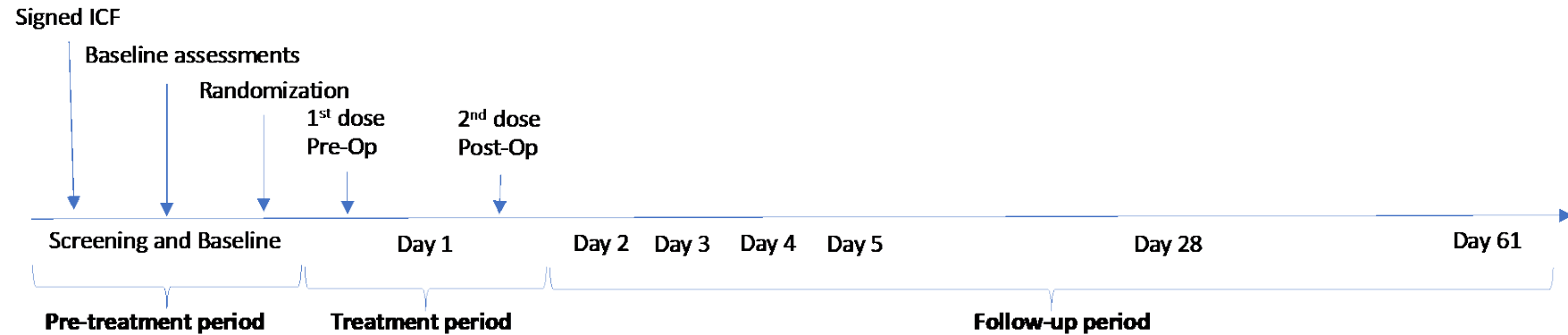
Sample size and power

With the proposed sample size of 150 (91.7% power to detect a 20% difference) to 250 (90.1% power to detect a 15% difference) the difference in sCreat ratio between treated and control patients can be detected with a one-sided alpha of 2.5%, assuming a mean of 1.28 sCreat ratio for the placebo arm and a pooled standard deviation of 0.465.

Flow chart and schedule of assessments

Figure 1: Flow chart for the individual patient

Trial flow for the individual patient from signing of informed consent until completion of the last visit



The trial consists of a pre-treatment period during which screening, baseline assessments and randomization will be performed, a treatment period (Day 1) during which the patient will receive two 1-hour continuous IV infusions of the investigational product (one pre-surgery and one post-surgery), and a follow-up period (Day 2 to Day 61) during which follow-up assessments will be performed.

Abbreviations: ICF = informed consent form, IV = intravenous, Op = operation/surgery.

Table 1: Schedule of assessments

	Pretreatment		Treatment		Follow-up					
	Screening	Baseline ¹	Surgery		Day 2	Day 3	Day 4	Day 5	Day 28* (±4d)	Day 61/ EoT (+10d)
	Day -30 to Day 1	Day -1/Day 1 (max 24 h before surgery ²)	pre-surgery	post-surgery						
Informed consent	X									
Inclusion/exclusion criteria ³	X	X ⁴								
Pregnancy test (urine or blood) ⁵	X	X ⁴								X
Follicle stimulating hormone ⁶	X									
Medical history ⁷	X									
Physical examination	X ⁸									
Demographics	X									
Weight, height		X ⁸								
EuroScore II		X								
12-lead ECG		X								
Prior and concomitant treatment	X	X ⁴		X	X	X	X	X	X	X ⁹
Randomization		X								
IP administration ¹⁰			X	X						
sCreat	X ¹¹	X ¹²		X ¹³	X	X	X	X		X
KDIGO AKI stage				X	X	X	X	X		
Blood sampling ¹⁴		X		X	X	X	X	X		X
Urine sampling ¹⁵		X		X	X	X	X	X		
Safety laboratory		X						X		(X ¹⁶)
PK samples ¹⁷				X	X	X	X	X		

Confidential

	Pretreatment		Treatment		Follow-up					
	Screening	Baseline ¹	Surgery		Day 2	Day 3	Day 4	Day 5	Day 28*	Day 61/ EoT
	Day -30 to Day 1	Day -1/Day 1 (max 24 h before surgery ²)	pre-surgery	post-surgery					(±4d)	(+10d)
Blood sample for ADA		X								X
RRT				X	X	X	X	X	X	X
Adverse events	X	X ⁴		X	X	X	X	X	X	X
Surgical parameters and complications ¹⁸			X	X	X	X	X	X	X	
ICU stay (start and end date, reason)				X	X	X	X	X	X	(X ¹⁹)
Mortality				X	X	X	X	X	X	X

* Can be a telephone visit.

1 All baseline assessments must be done before IP administration.

2 Start time of surgery is defined as first incision.

3 Patients may be rescreened 24 hours before surgery, if the patient does not fulfill the inclusion criterion 3 at the screening visit.

4 If Screening, Baseline and surgery are performed on the same day, the assessments do not need to be repeated (i.e., check of in-/exclusion criteria, prior treatment, pregnancy test, adverse events).

5 Women of childbearing potential only.

6 Only post-menopausal women.

7 Medical history will be collected by using the Charlson co-morbidity index supplemented with recent medical history relevant to cardiac surgery and potential kidney damage. In addition, any information on the history of the indication for the surgery should be collected.

8 If done as a standard of care on admission to hospital and within 72 hours prior to surgery, the physical examination, body weight and height do not need to be repeated at Baseline and data will be used for baseline assessment.

9 Only medication considered nephrotoxic and medication given for treatment of adverse events (s)/serious adverse event(s) must be documented up to Day 61.

10 The first dose of IP will be administered 6 to 0.5 hours before surgery (first incision), and the second dose of IP will be administered immediately after surgery, defined as start of infusion within 4 hours after closing of the chest. There should be at least 1 hour between end of first dose and start of second dose.

11 sCreat level, not older than 28 days, can be used for the eGFR calculation.

12 sCreat will be analyzed locally, for Baseline sCreat can be analyzed in the blood serum sample for safety laboratory.

Confidential

- 13 Blood for sCreat to be collected on Day 1 post-surgery after disconnection of the CPB, but prior to second dose of IP.
- 14 At Day 1 post-surgery the biomarker samples have to be taken after disconnection of CPB and before start of second dose. The sampling consists of a serum sample, a plasma EDTA sample and a plasma citrate sample.
- 15 Using spot sample for potential biomarker analysis.
- 16 Only in case of treatment discontinuation after first IP administration and trial termination prior to Day 5.
- 17 Blood for PK to be collected on Day 1 post-surgery (after disconnection of the CPB, but prior to second dose of IP), Day 2, Day 3, Day 4, and Day 5. Using central laboratory.
- 18 Includes peri-operative parameters, data and surgery related complications since Day 1. For the documentation of surgery related complications, the Society of Thoracic Surgeons national database initiative for adult cardiac surgery will be used as a reference.
- 19 Only in case of premature termination before Day 28.
- CBP = cardiopulmonary bypass pump, d = day(s), EoT = end of trial, EuroScore = European System for Cardiac Operative Risk Evaluation, IP = investigational product, sCreat = serum creatinine.

2 Addresses and responsibilities

Sponsor's signatory

[REDACTED]
AM-Pharma B.V.
Stadsplateau 7
3521 AZ Utrecht, The Netherlands
[REDACTED]

Sponsor's signatory

[REDACTED]
AM-Pharma B.V.
Stadsplateau 7
3521 AZ Utrecht, The Netherlands
[REDACTED]

Coordinating investigator

Prof Dr Peter Pickkers, MD, PhD
Radboud University Nijmegen Medical Centre
Geert Grooteplein Zuid 10
6525 GA Nijmegen, The Netherlands
Phone: +31 (0)243 615 363

Medical monitor

[REDACTED]
FGK Clinical Research GmbH
Heimeranstrasse 35
80339 Munich, Germany
Phone: +49 (0)89 893 119 224
Fax: +49 (0)89 893 119 20

Safety reporting

Fortrea Inc.
8 Moore Drive
Durham, North Carolina 27709, USA
Fax: +1 (0)800 664 8857

**Central laboratory for
pharmacokinetics and anti-drug
antibodies analysis**

ICON Bioanalytical Laboratories
Amerikaweg 18, 9407TK
Assen, The Netherlands
Phone: +31 (0)592 799 267

A complete list of study personnel will be available in the trial master file.

3 Table of contents

	Page
REVISION CHRONOLOGY	2
1 SUMMARY AND FLOW CHART	6
2 ADDRESSES AND RESPONSIBILITIES.....	17
3 TABLE OF CONTENTS	19
4 ABBREVIATIONS AND DEFINITION OF TERMS	24
5 INTRODUCTION.....	26
5.1 Major cardiothoracic surgery and acute kidney injury.....	26
5.2 Ilofotase alfa	27
5.3 Trial rationale.....	28
6 INVESTIGATORS, TRIAL ADMINISTRATIVE STRUCTURE, AND TRIAL COMMITTEES.....	29
6.1 Investigators and trial administrative structure.....	29
6.2 Trial steering committee	30
6.3 Data monitoring committee	30
7 TRIAL OBJECTIVES, ENDPOINTS, AND ESTIMANDS.....	30
8 TRIAL DESIGN AND DESIGN RATIONALE.....	33
8.1 Overall trial design.....	33
8.2 Trial design rationale.....	33
8.2.1 Rationale.....	33
8.2.2 Patient involvement in the trial design.....	34
8.3 Justification for dose	34
8.4 Risk/benefit assessment.....	35
9 PATIENT SELECTION	37
9.1 Sample size	37
9.2 Inclusion criteria	37
9.3 Exclusion criteria	38
9.4 Screening failures	39

10	MEASURES TO MINIMIZE BIAS: RANDOMIZATION AND BLINDING PROCEDURES	40
10.1	Patient identification	40
10.2	Randomization	40
10.3	Blinding and emergency unblinding.....	40
11	TREATMENTS.....	41
11.1	Investigational products.....	41
11.1.1	Description, packaging, and labeling	42
11.1.2	Storage and stability	42
11.1.3	Investigational product preparation, treatment dose, administration, and dispensing	43
11.1.4	Drug accountability and patient compliance	43
11.1.5	Medication errors and overdose.....	44
11.2	Previous and concomitant medication.....	44
11.2.1	Prohibited medications and concomitant medications to be avoided	45
11.3	General restrictions.....	45
12	TRIAL SCHEDULE	45
12.1	Trial conduct	45
12.2	Screening	46
12.3	Baseline.....	46
12.4	Treatment.....	46
12.5	Unscheduled visit.....	46
12.6	Follow-up.....	46
12.7	End of trial and early termination.....	47
12.8	Medical care upon termination of the clinical trial.....	47
12.9	Restrictions and contingency measures due to serious public health emergencies.....	47
13	EFFICACY ASSESSMENTS	48
13.1	Serum creatinine level.....	48
13.2	MAKE 60.....	48
13.3	KDIGO AKI stage.....	49
13.4	Length of intensive care unit stay after surgery.....	50

13.5	Biomarkers	50
14	PHARMACOKINETIC ASSESSMENTS	50
15	SAFETY ASSESSMENTS	51
15.1	Adverse events	51
15.1.1	Definitions	51
15.1.2	Classification of adverse events	53
15.1.3	Documentation of adverse events	54
15.1.4	Reporting of serious adverse events and suspected unexpected serious adverse reactions	55
15.1.5	Follow-up of adverse events	56
15.2	Pregnancy	57
15.3	Medication error and overdose	57
15.4	Laboratory investigations	58
15.5	Anti-drug antibodies	59
15.6	Physical examination and electrocardiogram	59
16	OTHER ASSESSMENTS	60
16.1	EuroScore II assessment	60
16.2	Peri-surgical factors and surgery related complications	61
17	STATISTICAL METHODS	63
17.1	Sample size calculation	63
17.2	General approach	63
17.3	Analysis sets and types of analyses	64
17.4	Handling of missing, unused, and spurious data	64
17.5	Analysis of trial conduct and patient disposition	65
17.6	Baseline characteristics	65
17.7	Efficacy analyses	65
17.7.1	Primary efficacy endpoint	65
17.7.2	Secondary endpoints	66
17.7.2.1	Efficacy	66
17.7.2.2	Safety	67
17.7.3	Exploratory endpoints	67
17.8	Pharmacokinetic	68

17.9	Safety analyses.....	68
17.9.1	Adverse events	68
17.9.2	Clinical laboratory.....	68
17.9.3	Anti-drug antibodies.....	69
17.10	Interim analysis	69
18	PATIENT WITHDRAWAL FROM TRIAL PARTICIPATION	69
19	ETHICAL AND LEGAL REQUIREMENTS.....	70
19.1	General requirements	70
19.2	Independent ethics committee or institutional review board	71
19.3	Patient recruitment and informed consent procedure.....	71
19.4	Insurance coverage.....	72
19.5	Submission to authorities.....	72
19.6	Patient confidentiality	72
20	CRITERIA FOR PREMATURE TERMINATION OF THE TRIAL AND FOR CLOSING A TRIAL CENTER	74
20.1	Criteria for terminating the clinical trial	74
20.2	Criteria for closing a trial center	74
21	TRIAL PROTOCOL, DOCUMENTATION AND ARCHIVING OF DATA.....	75
21.1	Modifications to the protocol.....	75
21.2	Protocol deviations	75
21.3	Data retention.....	76
22	DATA COLLECTION, MONITORING, AND QUALITY ASSURANCE.....	76
22.1	Data collection	76
22.2	Monitoring.....	77
22.3	Audits and inspection.....	78
22.4	Data management procedures	78
22.5	Training of center staff (optional).....	79
22.6	Serious breaches	79
23	TRIAL REPORT AND PUBLICATIONS	79
24	TRIAL PERIODS.....	80
25	REFERENCES	81

26	APPROVAL AND SIGNATURES	86
27	APPENDICES	88
APPENDIX 1	COMMON SURGICAL COMPLICATIONS	88

Table of Tables

Table 1:	Schedule of assessments	14
Table 2:	Objectives, endpoints, and estimands	31
Table 3:	Interventions used in the trial	42
Table 4:	Staging of acute kidney injury	50
Table 5:	Clinical safety and screening laboratory parameters	58

Table of Figures

Figure 1:	Flow chart for the individual patient	13
-----------	---	----

4 Abbreviations and definition of terms

ADA	anti-drug antibody
ADP	adenosine di-phosphate
ADR	adverse drug reaction
AE	adverse event
AKI	acute kidney injury
ANCOVA	analysis of covariance
AP	alkaline phosphatase
ATP	adenosine tri-phosphate
AUC	area under the curve
CA	competent authority
CABG	coronary artery bypass grafting
CPB	cardiopulmonary bypass pump
CSA	cardiothoracic surgery associated
CTIS	clinical trials information system
DAMP	damage-associated molecular pattern
DMC	data monitoring committee
ECC	endogenous creatinine clearance
ECG	electrocardiogram
eCRF	electronic case report form
eGFR	estimated glomerular filtration rate
EoT	end of trial
FAS	full analysis set
FSH	follicle-stimulating hormone
IABP	intra-aortic balloon counter-pulsation
ICF	informed consent form
ICU	intensive care unit
IEC	independent ethics committee
IP	investigational product
ISF	investigator site file
IV	intravenous
KDIGO	kidney disease improving global outcomes
LVAD	left ventricular assist device
MAD	multiple ascending dose
MAKE	major adverse kidney events

NGAL	neutrophil gelatinase-associated lipocalin
PAMP	pathogen associated molecular pattern
PD	protocol deviation
PK	pharmacokinetic(s)
PKS	pharmacokinetic set
PopPK	population pharmacokinetic
PPS	per-protocol set
RD	renal damage
recAP	recombinant human alkaline phosphatase
RRT	renal replacement therapy
SA	sepsis-associated acute kidney injury
SAD	single ascending dose
SAE	serious adverse event
SAF	safety analysis set
SAP	statistical analysis plan
sCreat	serum creatinine
SOP	standard operating procedure
STS	Society of Thoracic Surgeons
SUSAR	suspected unexpected serious adverse reaction
TEAE	treatment emergent adverse event
TSC	trial steering committee

Definitions

Trial start: The start of the trial is defined as the first act of recruitment of a potential patient for this trial.

5 Introduction

5.1 Major cardiothoracic surgery and acute kidney injury

Renal damage (RD), defined as persistent loss of renal function, is one of the most common, clinically important complications observed after open heart surgery in adult patients.[2,3,4,5,6] A key event contributing to this RD is acute kidney injury (AKI) immediately following the surgical procedure.[7] In open heart surgery performed with the use of a cardiopulmonary bypass pump (CPB), this AKI is thought to be the result of multiple factors, including inflammation, hemodynamic instability and disturbance of normal hemostasis.[4,8]

Up to ~30% of all patients (all-comers) scheduled for open heart surgery develop AKI after surgery, defined by an increase in serum creatinine (sCreat).[9] In patients with stage 3 chronic kidney disease (CKD; estimated glomerular filtration rate [eGFR] 30-60 mL/min/1.73 m²), the incidence of AKI increase up to ~85%.[10] While AKI requiring renal replacement therapy (RRT) is infrequent with an incidence between 2 and 5% of all patients undergoing cardiac surgery [11,12], significant mortality of up to 50% is reported when RRT is needed [13].

In response to this high proportion of patients suffering from RD associated with open heart surgery, risk factors for AKI have been identified and clinical scores developed to predict postoperative AKI [13] and associated long-term effects [10]. Prominent risk factors for both mortality and progression to end-stage renal disease are pre-existent renal impairment [14] and complexity of the surgical procedure associated with longer duration of CPB and cross-clamp time were especially relevant [15]. Consequently, the surgical procedure associated with the highest likelihood of AKI, and thus long-term RD, is open heart surgery with prolonged use of CPB with a cross-clamp time over 60 minutes, such as valve replacement, especially when combined with coronary artery bypass grafting (CABG), and complex CABG procedures. For the latter the cross-clamp time is directly related to the number of planned distal anastomoses. The likelihood of AKI increases further in patients who have pre-existing impaired renal function.

In AKI related to open heart surgery, there is a graded significant association of AKI with new-onset CKD during the first 3 years after surgery.[7,16] Furthermore, patients with a limited renal reserve are at increased risk for AKI and worsening of CKD as a consequence.[17,18] Therefore, it is hypothesized that prevention of AKI or reduction of AKI severity decreases the likelihood and impact of post-operative short- and long-term RD.[19,20,21]

At this moment specific pharmaceutical interventions are not yet available for preventive treatment of cardiothoracic surgery associated (CSA)-RD.

5.2 Ilofotase alfa

Ilofotase alfa, is a homodimeric recombinant human alkaline phosphatase (recAP). Alkaline phosphatase (AP) is an endogenous enzyme present in many cells and organs, e.g., intestines, placenta, liver, bone, kidney, and granulocytes. It exerts detoxifying effects through dephosphorylation of damage-associated molecular pattern (DAMP) molecules (e.g., adenosine tri- and di-phosphate [ATP and ADP]) and of pathogen associated molecular pattern (PAMP) molecules (e.g., endotoxins such as lipopolysaccharide). Systemic or local exposure to these DAMPs alert the organism to danger and tissue damage and lead to profound inflammation, amplified by massive chemoattraction and activation of innate immune cells, production of inflammatory cytokines and other mediators, platelet activation, and other sequelae. During major surgery where hemodynamic instability and hemostasis occur, the effect of DAMPs may be particularly evident in the kidney because of its blood filtration function.[\[22,23\]](#)

It is appreciated that tubular cells express toll-like receptor 4 and mount an inflammatory response following filtration of inflammatory mediators, such as DAMPs, and that renal inflammation and the subsequent microcirculatory dysfunction and negative effects on renal bioenergetics account for the tubular injury observed in cardiac surgery patients.[\[24\]](#) Therefore, dephosphorylation of pro-inflammatory ATP resulting in the formation of ADP, adenosine monophosphate, and eventually tissue-protective adenosine [\[23\]](#) may be more important in the kidney and especially in patients with decreased renal reserve. As a result, dephosphorylation by AP reduces the inflammatory responses and provides an anti-inflammatory effect.[\[23,25\]](#)

Interfering early in the inflammatory processes in the kidney is assumed to also exert effects later on through prevention of maladaptive repair processes, reducing severity and duration of AKI, reducing disease progression, specifically preventing interstitial fibrosis, hence maintaining better overall integrity of renal structures.[\[19,26\]](#) Preventive administration of ilofotase alfa in an elective setting in patients undergoing cardiac surgery is therefore expected to reduce incidence of post-operative CSA-AKI and thus prevent resulting CSA-RD.

Pre-clinical and clinical trials with ilofotase alfa

Pre-clinical studies with ilofotase alfa in rodent and porcine ischemia-reperfusion or systemic endotoxemia models showed that a single dose of ilofotase alfa consistently inhibited markers of inflammation and reduced kidney damage and functional impairment as assessed by sCreat, blood nitrogen, creatinine clearance and histopathological examination. Ischemia-reperfusion experiments with a CD73^{-/-} knockout mouse (not able to produce extracellular adenosine) showed that ilofotase alfa had protective effects through the generation of tissue -protective adenosine, which acts on the A2A adenosine receptor.

In two Phase 1 single ascending dose (SAD, in Part A) and multiple ascending dose (MAD, in Part B) clinical trials in 82 healthy volunteers [\[27\]](#), all reported adverse events (AEs) were

classified as mild or moderate and most were evenly distributed over the active and the placebo groups. Single intravenous (IV) infusion (in the range of 0.32-3.2 mg/kg) and multiple infusions of ilofotase alfa (at doses of 0.8 and 1.6 mg/kg for 3 consecutive days) did not raise any safety concerns and were well tolerated by healthy male and female subjects.

In a Phase 2a/2b proof-of-concept and dose-finding trial (STOP-AKI) in adult patients with sepsis-associated (SA)-AKI, an effect on both renal function (significant improvement of endogenous creatinine clearance [28]) after 28-days and mortality was observed in the 2 highest ilofotase alfa dose groups, 0.8 mg/kg and 1.6 mg/kg, compared to the placebo group, as well as a reduction in major adverse kidney events (MAKE) 90 in the 1.6 mg/kg treatment arm.[29] There were no safety or tolerability concerns for any of the doses tested (0.4, 0.8 and 1.6 mg/kg).[29] Pharmacokinetic (PK)/pharmacodynamic simulations confirmed the 1.6 mg/kg dose to have the most pronounced treatment effect. Most recently, ilofotase alfa was tested in a multinational Phase 3 clinical trial (REVIVAL) in patients with SA-AKI where it demonstrated an effect on MAKE, which is most pronounced in patients with pre-existent CKD. There were again no safety or tolerability concerns.

5.3 Trial rationale

In patients undergoing cardiac surgery, a relationship between AKI incidence, severity, duration and long-term loss of renal function has been demonstrated [30], and AKI results in both increased morbidity and mortality [31,32,33]. Importantly, this occurs even after very mild reductions in renal function as defined by the kidney disease improving global outcomes (KDIGO) stage 1.[34] Therefore, it is hypothesized that prevention or reduction of AKI influences the likelihood and impact of post-operative RD.[19,20,21]

Current management of CSA-AKI and the resulting CSA-RD does not include specific pharmaceutical interventions; trials with pharmaceutical interventions have proven to be challenging and limited in their success.[35,36,37,38,39,40]

While it is evident that acute major changes in renal function are deleterious, it has also been demonstrated that even a mild decrease in eGFR is associated with a higher mortality.[41,42]

Based upon the mechanism of action of ilofotase alfa in modulating DAMPs and PAMPs triggered by inflammation and ischemia that occur during and after cardiac surgery, together with the existing clinical data [29] and data generated in animal models of ischemia reperfusion injury [22] and CSA-AKI [43], it is appropriate to test whether treatment with ilofotase alfa will reduce the incidence and/or severity of AKI in patients undergoing complex elective cardiac surgery (CABG with 3 or more distal anastomoses or CABG combined with valve). The effect is assumed to be especially relevant for patients with already existing impaired renal function.

The pre-clinical and clinical data generated thus far underscore that the presence of ilofotase alfa during the renal insult as well as in the acute recovery period is optimal for protecting the kidney from both short- and long-term damage such as AKI, dialysis, and onset or progression of CKD.[22,43,44]

Based on literature [13,45,46] and the real-world evidence Study RAP-CLIN-0159 it is very clear that in cardiac surgery the two key risk factors for the development of AKI and consequently long-term RD are the presence of pre-existent limited renal function/CKD and the complexity and duration of surgery performed. In open chest on pump heart surgery, the complexity of surgery is essentially causing a longer need of the CPB and within this especially the cross-clamp time is the most significant factor, a cross-clamp time longer than 60 minutes is associated with more risk for complications including mortality.[15,47] During the period on CPB, and specifically the cross-clamp period, the renal homeostasis is most affected by blood pressure, the switch from pulsatile to continuous flow, disrupted clotting system, and high load of danger signals from the ongoing trauma and the post-operative inflammatory response. Studies indicate that especially these are contributing to the risk of AKI and subsequent RD.[22,35,43,44]

For patients scheduled for CABG without additional procedures, and eligible for the study, the cross-clamp time can prospectively only be estimated based upon the expected complexity of the planned procedure. In literature [47,48] and in discussions with cardiothoracic surgery experts and the trial steering committee, the number of planned distal anastomoses was recommended as the best proxy for the cross-clamp time, as being more predictive compared to, for example, the use of the internal mammary arteries or the total number of grafts. Another advantage is, it keeps the inclusion criteria simple and unambiguous which will support data quality and integrity.

In summary, it is estimated that a procedure with 3 or more distal anastomoses together with the proximal anastomose(s) requires a cross-clamp time of at least 60 minutes.

6 Investigators, trial administrative structure, and trial committees

6.1 Investigators and trial administrative structure

The trial will be conducted multicentric across Europe under the supervision of Prof Dr Peter Pickkers (coordinating investigator).

The sponsor, AM-Pharma B.V., is funding the trial and will be responsible for the overall supervision and administration of the trial. Monitoring, project management, regulatory submission, data management, statistical analysis, and medical writing will be done by FGK

Confidential

Clinical Research GmbH, Munich, Germany (in the following referred to as sponsor's designee). Safety reporting will be done by Fortrea Inc., Durham, USA.

Further vendors will be used for the following functions:

Electronic case report form (eCRF) by viedocTM (Uppsala, Sweden)

Central laboratory for PK and anti-drug antibodies (ADAs) analysis at ICON Bioanalytical Laboratories (Assen, The Netherlands)

6.2 Trial steering committee

The trial steering committee (TSC) will act as a communication platform between investigators, sponsor and sponsor's designee and will consist of the principle investigators of the trial centers, a representative of the sponsor, and the project manager at FGK. In preparation to and during the trial, regular telephone calls or web-meetings will be held under the leadership of the coordinating investigator to discuss trial-relevant topics.

6.3 Data monitoring committee

An independent data monitoring committee (DMC) consisting of independent experts in the fields of cardio thoracic surgery, critical care, and nephrology not otherwise involved in the trial will be established to continuously monitor the safety of the trial. The DMC will review and evaluate unblinded safety data including suspected unexpected serious adverse reaction (SUSAR) reports submitted to the authorities on an ongoing basis, and listings of related serious AEs (SAEs) on a monthly basis. If safety reports trigger any safety concerns, a DMC meeting can be initiated for further risk/benefit assessment and recommendation to the sponsor and TSC on trial continuation or discontinuation based on the safety reports reviewed. A charter including a description of responsibilities and the procedures for the assessments will be implemented before the data analysis.

7 Trial objectives, endpoints, and estimands

The following objectives and endpoints are defined in Table 2. The analysis of the primary objective, the sCreat ratio, and the secondary efficacy objective, MAKE 60, is based on the estimand concept. An estimand is specified by the definition of variable, treatment, target population, intercurrent events, and population-level summary. Estimands for the analysis are specified in [Table 2](#).

Table 2: Objectives, endpoints, and estimands

Objective	Endpoint and estimands								
Primary									
To assess the effect of ilofotase alfa on renal function	Endpoint/Variable: Ratio between the highest value post-surgery (post-surgery Day 1 and Days 2, 3, 4, and 5) and the pre-surgery baseline value for serum creatinine (sCreat) Treatment: The patients will be treated with the investigational product (IP; ilofotase alfa or placebo) at the same day before and after the surgery. The ilofotase alfa dose is a fixed dose of 128 mg per infusion. The matching placebo contains aqueous buffer supplied in glass vials that are identical to the ilofotase alfa vials in order to maintain the blind. Target Population: The target population consists of male and female adult patients scheduled for coronary artery bypass grafting (CABG) with 3 or more distal anastomoses or CABG combined with valve surgery that are at risk of developing renal damage based upon pre-surgery impaired kidney function (estimated glomerular filtration rate [eGFR] ≥ 25 and ≤ 65 mL/min/1.73m²). Intercurrent Events (ICEs) and Strategies: <table border="1"> <tr> <th>Intercurrent event</th><th>Strategy</th></tr> <tr> <td>1. Use of concomitant medications during surgery or until Day 5 which might influence the efficacy assessment</td><td>Treatment</td></tr> <tr> <td>2. Any deviation of the evaluation of sCreat (incorrect storage of sample, etc.)</td><td>Treatment</td></tr> <tr> <td>3. Death after second IP administration</td><td>Composite</td></tr> </table> The occurrence of ICEs 1 and 2 will be handled using the treatment policy strategy according to ICH guideline E9 (R1). All values will be used regardless of occurrence of the above mentioned intercurrent events during the study period. The occurrence of ICE number 3 will be handled using a composite strategy. If a patient dies after the second IP administration and before sCreat measurement on Day 5, the sCreat ratio will be set to 1.25, irrespectively of any measured sCreat values. Population-Level Summary: The sCreat ratio will be compared between ilofotase alfa and placebo by least squares (LS) means and LS means differences of an analysis of covariance with pre-surgery sCreat baseline value, eGFR stratification category, and type of surgery as covariates.	Intercurrent event	Strategy	1. Use of concomitant medications during surgery or until Day 5 which might influence the efficacy assessment	Treatment	2. Any deviation of the evaluation of sCreat (incorrect storage of sample, etc.)	Treatment	3. Death after second IP administration	Composite
Intercurrent event	Strategy								
1. Use of concomitant medications during surgery or until Day 5 which might influence the efficacy assessment	Treatment								
2. Any deviation of the evaluation of sCreat (incorrect storage of sample, etc.)	Treatment								
3. Death after second IP administration	Composite								

Objective	Endpoint and estimands						
Secondary							
Efficacy							
To assess the effect of treatment with ilofotase alfa on the clinical outcome in patients undergoing cardiac surgery	Endpoint variable: Major adverse kidney events (MAKE) 60, defined as died up to and including Day 61, have received or are receiving new renal replacement therapy up to and including Day 61, or have a decrease in eGFR $\geq 25\%$ on Day 61 compared to the pre-surgery eGFR reference value Treatment and Target Population: analogue primary endpoint Intercurrent Events (ICEs) and Strategies: <table border="1"> <thead> <tr> <th>Intercurrent event</th><th>Strategy</th></tr> </thead> <tbody> <tr> <td>1. Use of concomitant medications during surgery or until Day 5 which might influence the efficacy assessment</td><td>Treatment</td></tr> <tr> <td>2. Any deviation of the evaluation of sCreat (incorrect storage of sample, etc.)</td><td>Treatment</td></tr> </tbody> </table> The occurrence of ICEs 1 and 2 will be handled using the treatment policy strategy defined according to ICH guideline E9 (R1). All values will be used regardless of occurrence of the above mentioned ICEs during the study period. Population-Level Summary: The proportion of patients with MAKE 60 (yes/no) will be compared between ilofotase alfa and placebo by means of a logistic regression approach with pre-operative sCreat baseline value, eGFR stratification category, and type of surgery stratification category as covariates.	Intercurrent event	Strategy	1. Use of concomitant medications during surgery or until Day 5 which might influence the efficacy assessment	Treatment	2. Any deviation of the evaluation of sCreat (incorrect storage of sample, etc.)	Treatment
Intercurrent event	Strategy						
1. Use of concomitant medications during surgery or until Day 5 which might influence the efficacy assessment	Treatment						
2. Any deviation of the evaluation of sCreat (incorrect storage of sample, etc.)	Treatment						
Safety							
To assess the safety and tolerability of ilofotase alfa in patients undergoing open chest heart surgery	Incidence rate of adverse events (AEs) and serious AEs through Day 28						
Exploratory							
- To investigate incidence, duration, and severity of acute kidney injury (AKI) - To assess the effect on surgery related complications	- The area under the curve of AKI (kidney disease improving global outcomes sCreat), defined as duration (in days) and severity of AKI; measured over the first 5 days after surgery - KDIGO AKI incidence over the 5 days after surgery - Postoperative surgery related complications within 28 days of surgery, assessed according to the Society of Thoracic Surgeons definition						

Objective	Endpoint and estimands
- To assess the duration of critical care after the scheduled surgery - To investigate biomarkers of AKI (optional after the end of the trial)	- Length of stay in the intensive care unit in days after the initial surgery - Changes in biomarkers of AKI compared to Baseline
Pharmacokinetics	
To investigate PK properties of ilofotase alfa	Population (Pop)PK

8 Trial design and design rationale

8.1 Overall trial design

This is a Phase 2, multi-center, randomized, double-blind, placebo-controlled, 2-arm parallel group design trial in patients at risk for RD following open heart surgery.

The clinical trial consists of a pre-treatment period of up to 30 days, a treatment period of 1 day and a follow-up period of 60 days. The total duration of trial participation for a patient will be about 3 months.

After screening and baseline assessments, eligible patients will be randomized 1:1 to either 2 doses of 128 mg ilofotase alfa, or 2 doses of placebo control. Randomization will be stratified by baseline eGFR (≤ 45 mL/min/m² or >45 mL/min/m²) and type of surgery (CABG with 3 or more distal anastomoses or CABG combined with valve surgery or aortic valve plus aortic root and/or ascending aorta (excluding aortic arch). The patient will be treated with the investigational product (IP; ilofotase alfa or placebo) at the same day before and after the surgery (Day 1). Follow-up clinic visits will be performed daily up to Day 5 for efficacy, safety, and PK assessments. A safety follow-up can be performed by telephone at Day 28 (± 4 days). At Day 61 (+10 days), an end of trial (EoT) visit will be done at site for efficacy, safety, and ADAs assessments. All trial activities are shown in the schedule of assessments ([Table 1](#)).

8.2 Trial design rationale

8.2.1 Rationale

A placebo arm is included due to regulatory recommendations to evaluate drug related benefits and adverse effects in randomized, double-blind, placebo-controlled trials. Because rescue medication is allowed during the entire trial, a placebo arm is justified.

Risk reduction of RD after complex cardiac surgery with long CPB in patients with high risk for RD after cardiac surgery due to pre-existent CKD is believed to be critical for treatment success and prevention of later complications such as RRT and long-term preservation of organ

function. It is therefore of utmost importance to preventively treat patients at risk of RD after cardiac surgery. In this trial, patients who will undergo complex elective cardiac surgery will be screened for their risk of developing RD. Only patients with a planned surgery procedure where the cross-clamp time is likely to be longer than 60 minutes and a pre-operative kidney function of $\text{eGFR} \geq 25$ and $\leq 65 \text{ mL/min/1.73m}^2$ will be enrolled in the trial. Additionally, the KDIGO AKI stage will be assessed at each after surgery visit using sCreat to identify CSA-AKI. The change in sCreat ratio between the highest value post-surgery (post-surgery Day 1 and Days 2, 3, 4 and 5) and the pre-surgery baseline value for sCreat is defined as the primary endpoint to assess the effect of ilofotase alfa on renal function.

To capture the long-term effects of AKI, MAKE has been proposed as an endpoint in clinical trials. MAKE is a composite endpoint consisting of mortality, new RRT, and a significant decrease in eGFR.[10,49,50] MAKE has proven to be valuable in interventional and follow-up trials. In interventional trials in patients with diabetic nephropathy, persistent changes in eGFR are considered a suitable endpoint to capture loss of kidney function in patients with an impaired kidney function.[51,52] A decrease in eGFR 60 days after surgery is very likely related to long-term reduction of renal reserve and increased mortality.

The trial population defined by the eligibility criteria (Section 9) is considered to be homogenous enough to observe potential drug effects but not to restrict the external validity of the trial results. Randomization of patients will be stratified by baseline eGFR ($\leq 45 \text{ mL/min/m}^2$ or $>45 \text{ mL/min/m}^2$) and type of surgery (CABG with 3 or more distal anastomoses or CABG combined with valve surgery) to get an equal distribution in the trial population. The distribution of male and female patients will be according to the natural distribution within the trial population, expecting more male than female patients. No corrective measures will be taken.

8.2.2 Patient involvement in the trial design

Patients are not involved in trial design.

8.3 Justification for dose

The dose regimen in this planned CSA-RD prevention trial (AP-recAP-CSA-RD-02-01) is supported by non-clinical data and clinical data in healthy volunteers and patients with SA-AKI. Adult patients scheduled for CABG who are at risk of developing RD based upon pre-operative impaired kidney function will be treated with 2 IV doses of ilofotase alfa or placebo (one pre-operative and one post-operative on the same day). A fixed dose of 128 mg ilofotase alfa (corresponding to a dose of 1.6 mg/kg for an 80 kg patient) is planned per IV administration. A flat dose of 128 mg administered intravenously is considered to reduce potential dosing errors, provide convenience in drug administration in this clinical setting, and to reduce wastage. A

population (Pop)PK model was applied to the Phase 2 trial STOP-AKI and the Phase 3 trial REVIVAL data and predicted similar exposures for patients regardless of weight, hence with a flat dose of 128 mg administered intravenously, a body weight <80 kg is not expected to substantially increase exposure. Lower body weights are therefore not anticipated to influence safety outcomes under the proposed flat dosing regimen. This is supported by two Phase 1 SAD/MAD trials in healthy volunteers, where single IV doses of up to 3.2 mg/kg did not raise any safety or tolerability concerns. A putatively higher peak exposure, due to a second dosing within 24 hours, will remain below the peak exposures observed in Phase 1 trials. Patients weighing ≤ 55 kg will be excluded from the trial. In the completed Phase 3 trial in SA-AKI, the 1.6 mg/kg IV dose had favorable effects on lowering MAKE 90 in a CKD population. Thus, this dose is expected to have reno-protective effects in cardiovascular surgery. More details are provided in the investigator's brochure.

We have included continuous monitoring of SUSARs, and SAE on a monthly basis by the DMC to be maximally informed on the safety profile of the flat dose in the studied patient population.

8.4 Risk/benefit assessment

In two Phase 1 SAD and MAD trial [53], 62 of 82 healthy volunteers subjects were exposed to single doses of up to 3.2 mg/kg ilofotase alfa or daily doses of 0.8 mg/kg or 1.6 mg/kg administered for 3 consecutive days. No SAEs were observed and there were no clinically significant findings from clinical laboratory, 12-lead electrocardiogram (ECG), continuous cardiac monitoring or physical examination during the trials. All treatment emergent AEs (TEAEs) were transient and had resolved without sequelae by the follow-up period. One event of nausea in the 3.2 mg/kg dose group (SAD) was reported as possibly related to the trial drug and leading to treatment discontinuation. The overall percentage of subjects reporting TEAEs was generally comparable in the placebo and ilofotase alfa groups. Furthermore, the number of TEAEs did not increase with increasing dose. In the 3.2 mg/kg ilofotase alfa group of Part A (SAD), 1 subject had a confirmed positive result for ADA on Day 1, but the subsequent results (Day 9 and follow-up) were negative. In the 1.6 mg/kg ilofotase alfa group of Part B (MAD), 1 subject had confirmed positive results for ADA on Day 13 and follow-up with a titer of 1 (not positive after a fourfold dilution).

In the STOP-AKI [29,54], patients with SA-AKI were treated with 3 doses of ilofotase alfa (0.4, 0.8 and 1.6 mg/kg) or placebo. A total of 182 patients with SA-AKI were exposed to ilofotase alfa. One patient in the 0.4 mg/kg group discontinued from the trial drug treatment due to a non-serious TEAE of severe hypoxia. The patient was a 49-year-old Caucasian male with SA-AKI who was randomly assigned to the ilofotase alfa 0.4 mg/kg treatment group. The event of hypoxia was considered not recovered/not resolved when the patient discontinued from

the trial. The investigator assessed the event of hypoxia to be unrelated to the trial drug. In general, hypoxia was observed incidentally with no difference between dosing groups.

Serious TEAEs were reported in 43.1% and 50.0% of patients in the 1.6 mg/kg ilofotase alfa and placebo groups, respectively, reporting a total of 76 and 89 serious TEAEs, respectively. The occurrence of non-serious TEAEs was comparable between the 1.6 mg/kg ilofotase alfa and placebo groups and the majority of TEAEs were considered by the investigator to be unrelated to trial drug. The percentage of TEAEs considered by the investigator to be possibly related to trial drug was 20.2% and 13.4% in the 1.6 mg/kg ilofotase alfa group and placebo group, respectively. Most TEAEs were mild or moderate in severity. Fatal SAEs were reported for 29.5%, 26.3%, 17.1%, and 17.4% patients in the placebo, 0.4 mg/kg, 0.8 mg/kg, and 1.6 mg/kg ilofotase alfa groups, respectively, indicating a potential benefit on survival.

Post-hoc analyses of ECC resulted in a higher ECC in the treatment group compared to placebo (mean difference of 27.6 mL/min [95% CI = 8.7 to -46.6; $p = 0.004$]) for Day 21 and a mean difference of 18.2 mL/min [95% CI = -2.5 to 38.9; $p = 0.08$] for Day 28). These improvements in ECC at 1.6 mg/kg in the ilofotase alfa group compared to the placebo group indicated a potential benefit on long-term kidney function.

These clinical trials conducted to date showed that ilofotase alfa is safe and well-tolerated with potential clinical benefits observed in the Phase 2 trial (STOP-AKI).

In a Phase 3 trial (REVIVAL), patients with SA-AKI were randomly assigned to receive 1.6 mg/kg ilofotase alfa or matching placebo as a 1-hour IV infusion on 3 consecutive days. IP was given as add-on to standard of care. The trial was terminated early per recommendation of DMC after planned futility analysis on the primary endpoint, 28-day mortality involving the initial 411 patients, without any safety concern. Data from all 650 patients who were included in REVIVAL trial combined population prior to termination, consisting of 556 patients in the main trial population, 61 patients with moderate-to-severe CKD and 33 patients with COVID-19, with all patients having sepsis and AKI, were analysed. A lower proportion of patients receiving 1.6 mg/kg ilofotase alfa were estimated to experience MAKE 90 events (death, need for dialysis, substantial kidney function deterioration [$>25\%$ decline in eGFR] by Day 90, and rehospitalization) after the start of trial drug compared with patients receiving placebo (57.2% for ilofotase alfa and 64.7% for placebo; $p < 0.05$). This effect was significantly related to baseline renal function and was more pronounced in patients with increasing impaired pre-existent renal function. These results suggested that the therapeutic efficacy of ilofotase alfa was more pronounced in patients with a lower pre-AKI eGFR, i.e., more severe pre-existent CKD. Additionally, in the COVID-19 cohort of the trial, patients treated with ilofotase alfa experienced a reduction in 28-day all-cause mortality compared to those treated with placebo ($p < 0.01$). More specifically, patients in the ilofotase alfa group had 98% reduced odds of dying by Day 28 compared to those in the placebo group.

To date, a total of 62 healthy volunteers and 512 SA-AKI patients have been exposed to ilofotase alfa during Phase 1, 2 and 3 trials. The risk profiles from these trials are favorable.

Risk minimization procedures are implemented for this trial to minimize risks to patients. Specific eligibility criteria will ensure that patients who present with characteristics that may increase the risk for an adverse outcome are excluded. The occurrence of AEs will be monitored throughout the trial. The amount of blood collected will be strictly controlled and the trial participants will receive standard of care at the site by trained professionals, if needed.

Considering the favorable safety and tolerability profile observed in the Phase 1, 2 and 3 trials, marked clinical benefits on survival and improved long-term kidney function observed in the Phase 2 trial and significant reduced proportion of MAKE 90 in the Phase 3, the risk minimization measures, the risk-benefit evaluation is considered favorable.

9 Patient selection

9.1 Sample size

Up to 250 patients are planned to be enrolled so that up to 125 patients per treatment group will be randomized. The sample size calculation is described in Section 17.1.

9.2 Inclusion criteria

Patients meeting the following criteria will be considered for inclusion into the trial:

1. Age ≥ 18 years
2. Planned for one of the following types of open chest cardiovascular surgery with the use of CPB:
 - a. 1) combined valve and CABG surgery
 - 2) aortic valve plus aortic root and/or ascending aorta (excluding aortic arch)
 - b. CABG with 3 or more distal anastomoses
3. Screening eGFR ≥ 25 mL/min/1.73m² and ≤ 65 mL/min/1.73m² (calculated at Screening using sCreat which can be maximally 28 days old and the 2021 CKD-EPI formula [1] without race correction (eGFR = $142 \times \text{Min}(\text{Scr}/\kappa, 1)^\alpha \times \text{Max}(\text{Scr}/\kappa, 1)^{-1.200} \times 0.9938^{\text{Age}}$ [$\times 1.012$ if female])¹

¹ Scr is serum creatinine (mg/dL), κ is 0.7 for females and 0.9 for males, α is -0.241 for females and -0.302 for males, Min indicates the minimum of Scr/ κ or 1, and Max indicates the maximum of Scr/ κ or 1.

4. Female patients of childbearing potential agreeing to use one of the following effective contraception methods within IP treatment and 14 days thereafter:
 - a. combined (oestrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation (oral, intravaginal, transdermal)
 - b. progestogen-only hormonal contraception associated with inhibition of ovulation (oral, injectable, implantable)
 - c. intrauterine device
 - d. intrauterine hormone-releasing system
 - e. bilateral tubal occlusion
 - f. vasectomized partner (with medical assessment of the surgical success)
 - g. sexual abstinence

Abstinence is only accepted as true abstinence when this is in line with the preferred and usual lifestyle of the patient. Periodic abstinence (e.g., calendar, ovulation, symptothermal, post-ovulation methods and withdrawal) is not an acceptable method of contraception.

Post-menopausal females (i.e., >60 years of age or no menses for 12 consecutive months without an alternative medical cause with confirmatory follicle-stimulating hormone (FSH) level of ≥ 40 mIU/mL) do not require contraception during the trial.

5. Male patients agreeing to refrain from donating sperm, use a male condom when having sexual intercourse and in case their partner is of childbearing potential they must agree to use adequate and effective contraception method (see inclusion criterion 4) within IP treatment and 14 days thereafter

9.3 Exclusion criteria

A patient will not be eligible for inclusion if any of the following criteria applies:

1. Body weight ≤ 55 kg
2. Known or suspected glomerulonephritis (other than diabetic kidney disease) or other systemic vasculitis
3. Confirmed or treated endocarditis requiring antimicrobial or antiviral treatment within 30 days prior to surgery or other current active infection requiring antimicrobial or antiviral treatment within 14 days prior to surgery
4. Known chronic liver disorder with Child-Pugh C classification
5. Current planned (or scheduled) surgical intervention under conditions of circulatory arrest, including planned deep hypothermic circulatory arrest
6. Planned surgery for aortic dissection
7. Use of left ventricular assist device (LVAD), or intra-aortic balloon pump or other cardiac devices, within 7 days prior to surgery

8. Received any investigational drug within 30 days or 5 half-lives (whichever is longer) prior to Day 1 or planned or current participation in another interventional study until the EoT visit has been completed
9. Previous receipt of ilofotase alfa
10. Not willing and able to understand the information on the nature, the scope, and the relevance of the trial and to provide voluntary, written informed consent to participate in the trial before any trial related procedures
11. Pregnant or nursing women
12. Known or suspected hypersensitivity to ilofotase alfa or any components of the formulation used
13. Any previous organ transplantation
14. Congenital heart disease
15. Diagnosed with AKI (as defined by KDIGO criteria) within 3 months prior to surgery
16. History of RRT
17. Cardiogenic shock or hemodynamic instability which require inotropes or vasopressors or other mechanical devices such as intra-aortic balloon counter-pulsation (IABP) within 24 hours prior to surgery
18. A requirement for any of the following within one week prior to surgery: implantation of a defibrillator or permanent pacemaker, mechanical ventilation, IABP, LVAD, other forms of mechanical circulatory support
19. Required cardiopulmonary resuscitation within 14 days prior to cardiac surgery
20. Ongoing sepsis (as defined by SEPSIS-3, the Third International Consensus Definitions for Sepsis and Septic Shock) within the past 2 weeks or, in the opinion of the investigator, an untreated diagnosed clinically significant infection (viral or bacterial) prior to or at Screening and before randomization
21. Medical condition which requires active immunosuppressive treatment (daily steroid doses of ≤ 10 mg are allowed)
22. Employees or relatives of the sponsor or the investigator

9.4 Screening failures

Patients who do not meet the eligibility criteria for participation in this trial will be considered screening failures and will not subsequently be randomly allocated to IP. The reason will be recorded in the eCRF. Patients may be rescreened if the screening failure was not due to an ongoing medical condition. Patients may also be rescreened 24 hours before surgery if the patient does not fulfill the inclusion criterion 3 at the screening visit.

Rescreened patients will be assigned a new patient number.

10 Measures to minimize bias: randomization and blinding procedures

10.1 Patient identification

A unique patient number will be assigned to each patient who signed the informed consent form (ICF). The patient number will be assigned via eCRF and consists of 3 digits for international telephone code, 3 digits for site and 4 digits for the patient.

10.2 Randomization

Eligible patients will be randomly assigned in a 1:1 ratio ilofotase alfa 128 mg or placebo by means of a computer-generated randomization list.

The patients will be randomized stratified by baseline eGFR (≤ 45 mL/min/m² or >45 mL/min/m²) and type of surgery (CABG with 3 or more distal anastomoses or CABG combined with valve surgery) in the eCRF. In the proposed study cohort of 250 patients it is expected that approximately 187 patients (~75%) will have a baseline eGFR >45 mL/min/m² and that approximately 175 patients (~70%) will have CABG with 3 or more distal anastomoses (Cerner™ database and Study RAP-CLIN-0159). The automated system will assign the appropriate IP to each patient.

10.3 Blinding and emergency unblinding

Blinding

During the entire trial, patients, investigators, the sponsor, and all other persons involved in the conduct of the trial will be blinded to treatment. To maintain the blind, the ilofotase alfa 128 mg and the placebo concentrate for preparing a solution for infusion will have identical appearance, shape, and color, and will have identical labeling and packaging. In addition, filters for IP administration are provided by the sponsor to maintain the blind. To minimize the potential for bias, treatment randomization information will be kept confidential by the responsible personnel and will not be revealed until all patients have completed the trial and the data base lock has been performed.

The unblinded outputs for the safety data review by the DMC will be provided by Fortrea Inc. to an unblinded DMC support person at FGK not otherwise involved in the trial, who will forward them to the DMC members.

To avoid unblinding of the trial team, formal approved site-specific blinding plans need to be in place before the initiation visit to prevent measuring and/or reporting of AP activity plasma levels as part of the routine clinical chemistry panel. This measure must be in place up to and

including Day 14, irrespective if the patient is in the intensive care unit (ICU), in the intermediate care unit or in the general ward. Measuring and reporting AP activity could not only lead to unblinding of staff but might also lead to erroneous interpretation of liver function, as administration of ilofotase alfa will lead to increased AP activity levels, to values significantly exceeding the upper limit of the reference normal range. For that reason, AP activity levels should not be measured from samples obtained during the first 14 days of the trial. If measured as part of a standard package, the results should not be reported. Any reporting of AP activity levels is considered an important protocol deviation (PD). After Day 14, AP activity levels are expected to have returned to normal considering the elimination half-life of ilofotase alfa (~59 hours). Results of AP activity in samples taken after Day 14 may be reported.

PK data (other than date and time of collection) will be maintained by personnel unblinded to the trial for this purpose, but not involved in the trial otherwise.

Full details of the handling of restricted and potentially unblinding data will be documented.

Emergency unblinding

The premature unblinding of patient's IP assignment should be restricted to emergency cases in which knowledge of the administered drug is necessary for adequate treatment. The investigator is responsible to decide if such an emergency is present. The investigator must contact the medical monitor to discuss the medical emergency and the reason for revealing the actual treatment unless immediate unblinding is necessary to ensure the patient's safety.

Emergency unblinding, if necessary, will be performed via the eCRF. The investigator will be provided with technical options and password information to selectively break the code for an individual patient.

11 Treatments

11.1 Investigational products

IPs supplied by the sponsor (see Table 3) will be manufactured, tested, and released according to current Good Manufacturing Practice guidelines. Ilofotase alfa is not authorized so far.

Table 3: Interventions used in the trial

Intervention name	Ilofotase alfa	Placebo
Type	Drug	Drug
Dose formulation	Clear, colorless to slightly yellow concentrate for infusion	Clear, colorless to slightly yellow concentrate for infusion
Ingredients	[REDACTED]	[REDACTED]
Dosage	128 mg once before and after surgery at Day 1	Once before and after surgery at Day 1
Route of administration	Intravenous infusion	Intravenous infusion
IP and AxMP	IP	IP
Sourcing	Provided centrally by the sponsor	Provided centrally by the sponsor
Packaging and labeling	Provided in one pack per patient containing 8 vials. The vials are 8 mL type 1 glass vials with a FluroTec coated chlorobutyl rubber stopper aluminum crimping cap and a plastic flip-off overcap. Vial contains 4 mL of extractable ilofotase alfa 8 mg/mL solution. Each pack and glass vial will be labeled as required per country requirement.	Provided in one pack per patient containing 8 vials. The vials are 8 mL type 1 glass vials with a FluroTec coated chlorobutyl rubber stopper, aluminum crimping cap and a plastic flip-off overcap. Vial contains 4 mL of extractable placebo solution. Each pack and glass vial will be labeled as required per country requirement.
Manufacturer	[REDACTED]	[REDACTED]

11.1.1 Description, packaging, and labeling

IPs will be packed and labelled according to applicable regulatory requirements.

11.1.2 Storage and stability

The investigator is responsible for safe and proper handling and storage of the IP at the investigational site according to the labeled storage conditions. The IP must be stored temperature monitored at 2°C to 8°C and protected from light and moisture until preparation for use in a locked facility with access limited to the investigator and authorized personnel.

11.1.3 Investigational product preparation, treatment dose, administration, and dispensing

Ilofotase alfa and matching placebo will be provided in one treatment pack per patient containing one box with eight vials covering both infusions. Each 8 mL vial has an extractable volume of 4 mL.

Prior to IP administration, the four vials, with a total of 16 mL IP will be diluted with sterile sodium chloride 0.9% for injection (isotonic saline), USP/EP or equivalent, to a final volume of 50 mL. The ilofotase alfa dose is 128 mg.

Reconstituted IP can be stored at 2 to 8°C for up to 24 hours or alternatively for a maximum of 8 hours at room temperature. If applicable, the IP will be re-labelled (as per current regulations) by the hospital pharmacy with a new expiration date. Additional details will be provided in the Pharmacy Manual.

Reconstituted IP will be administered as an IV infusion, using a dosing syringe or infusion bag, an infusion line and filter, via a central catheter (preferred route) or a peripheral line, separately from any other concomitant medication via a dedicated lumen of the catheter or a separate peripheral line. Two IV infusions will be given at Day 1. The IP must be administered pre-surgery (up to 6 hours before, started at least 30 minutes before first incision) and immediately post-surgery at Day 1 (at least within 4 hours after closing the chest) by 1-hour continuous IV infusion with at least 1 hour between completion of the first administration and the start of the second administration.

If IP administration is interrupted for any reason, re-starting within 8 hours is encouraged as soon as possible and completed as scheduled and documented in the source documents. If it is not possible to restart within 8 hours, the remaining volume in the syringe should be documented in the source documents and the remaining drug should be discarded. The reason for and date of IP discontinuation and, if applicable, the discarded amount must be recorded in the source documents and eCRF.

To assure the use of compatible materials throughout the trial, syringe, infusion line, and filters for IP administration are provided by the sponsor.

11.1.4 Drug accountability and patient compliance

The IP must not be used outside the context of this trial protocol. The investigator must ensure that the IP is administered only to the patients enrolled in this trial. The investigator or authorized staff will record the correct receipt of IP at the center.

The IP will be administered by the investigator or qualified trial staff. Date and start and stop time of each IP administration as well how much IP was dispensed will be entered in the eCRF.

Because IP will not be handed out to the patients, no further measures to assess compliance will be undertaken.

Deviations from the dosage or dispensing regimen will be recorded in the eCRF.

11.1.5 Medication errors and overdose

In healthy volunteers, the maximum administered single dose was 3.2 mg/kg and this dose did not result in any SAEs. The maximum multiple dose that was administered to healthy volunteers was 1.6 mg/kg per day for 3 days, also without any SAEs.

To maximize both compliance to the protocol and minimal disruption of the routines at the site, the dosing needs to be started the earliest 6 hours before and the latest 30 minutes before the first incision. Based on the known PK, this will warrant adequate presence of study medication during the surgery itself. The 2nd dose aimed to protect during the acute recovery period should be given at completion of the surgery defined as closure of the sternum with a window of 4 hours with a minimum of 60 minutes between completion of the 1st dose and start of the 2nd dose, again to warrant compliance with local procedures. In case of non-compliance to the per protocol described interval of 60 minutes, based upon literature [55,56], the feedback from the cardiothoracic experts, and the investigators, the absolute minimal time span between first incision and closure of the sternum is 60 minutes (on average this was 3.5 hours in similar cohorts [36,55]). This results in minimally 30 minutes between completion of the 1st infusion with study medication and start of the 2nd infusion in those patients that were started the latest allowed before surgery and dosed at closing of the chest. In those patients the peak exposure will still be consistent with the 3.2 mg/kg dose as tested in healthy volunteers since separating the 3.2 mg/kg dose into 2 times 1.6 mg/kg doses with any time separation leads to a reduction of C_{max} by definition. In addition, no relation between AEs and SAEs and the observed plasma concentrations of ilofotase alfa in the completed clinical studies was found.

No specific treatment is available for medication errors including overdose (i.e., no reason to unblind) and there are no indications that specific symptoms will occur. If a medication error occurs, as a general rule, supportive care and treatment of symptoms must be provided.

Symptoms resulting from an IP overdose should be treated as medically indicated. Trial management should be contacted to discuss the details of overdose.

11.2 Previous and concomitant medication

All patients in this trial will receive supportive care according to best practice. The management of a patient should be based on the KDIGO Clinical Practice Guidelines for AKI.[31] Concomitant medication may be administered, and procedures may be conducted for the well-being of the patient at the discretion of the investigator.

All medication administered during Screening up to IP intake are regarded as previous medication. All treatments being taken by the patients from start of IP administration on Day 1 to Day 61 are regarded as concomitant treatments.

Medication considered nephrotoxic and medication given for treatment of AE(s)/SAE(s) must be documented from Screening up to Day 61.

Standard peri operative medication and medication given as standard of care during the hospital stay (e.g., blood pressure drugs, analgesics, anti-arrhythmia, anesthetics, analgesics, muscle-relaxants, etc.) will be reported in a categorized way.

In case of an AE or SAE, all concomitant medication of relevance to the event must be entered into eCRF. The medical monitor must be contacted if there are any questions regarding concomitant or prior medications.

Vasopressors, and inotropes must be collected from the start of the surgery until the end of the hospital stay and documented in the eCRF. The application of RRT (any modality) should be recorded as well, for RRT definition see details in Section 13.2.

11.2.1 Prohibited medications and concomitant medications to be avoided

After randomization, the administration of nephrotoxic drugs, such as contrast agents, non-steroidal anti-inflammatory drugs, angiotensin-converting enzyme inhibitors, gentamycin, or tobramycin should be avoided during trial participation where possible, as recommended by the KDIGO Clinical Practice Guideline for AKI recommendations. When used, details of dose and treatment duration should be recorded.

11.3 General restrictions

There are no restrictions on meal, diet or activity, and no restrictions on caffeine, alcohol and tobacco use during trial participation.

12 Trial schedule

12.1 Trial conduct

An overview on trial conduct is provided in the schedule of assessments (Table 1). All trial visits are generally scheduled as on-site visits. The Day 28 visit during the follow-up can be performed as telephone visit. Screening, Baseline and surgery can be performed on the same day and the assessments applicable to Screening and Baseline do need to be performed only once.

12.2 Screening

Patients for whom written informed consent was obtained (for consent procedures see Section 19.3) will be screened for eligibility based on the trial's inclusion and exclusion criteria (Sections 9.2 and 9.3, respectively) within 30 days before the day of the planned cardiac surgery and undergo the assessments shown in Table 1. The patients are considered enrolled at the time of signing the ICF. If the surgery is postponed for non-medical reasons, the time window is extended from 30 to 60 days.

12.3 Baseline

Baseline assessments will take place on Day -1 or Day 1, before IP administration and surgery, and must be performed within 24 hours prior to the start of the surgery (i.e. first incision). During this visit, all baseline assessments will be performed as outlined in Table 1, and the patient will be randomly allocated to a treatment group.

12.4 Treatment

IP administration will start with the first dose administered before surgery after randomization and end with a second dose administered after completion of the surgery on Day 1 (further details are provided in Section 11.1.3).

Pre- and post-surgery assessments will be performed as outlined in Table 1.

12.5 Unscheduled visit

Unscheduled visits may be performed at any time. If an unscheduled visit is performed, it should be documented whether a patient is on RRT. Other assessments may be performed at the investigator's discretion.

12.6 Follow-up

During the follow-up, the assessments outlined in Table 1 will be performed.

The follow-up includes 6 visits. Day 2 visit is defined by the time of the sCreat sampling post-surgery and will be conducted 24 hours after the start of the surgery and followed by 24-hour intervals for each visit on Day 3, 4 and 5 with ± 2 -hour time window. During hospitalization, daily visits will be performed up to Day 5. One month after IP administration, the Day 28 (± 4 days) visit can be done by a telephone call. The follow-up will end after 60 (+10) days on Day 61 with a EoT visit at the trial center.

When a patient is suspected lost to follow-up, the investigator or designee must make the following efforts to regain contact with the patient or his legal representative (a minimum of

3 telephone calls and, if necessary, a certified letter to the patient's last known mailing address or local equivalent methods or a home visit by a third vendor, if allowed by local regulations, and the final call on Day 61 (+10 days). The patient, patient's legal representative, patient's primary care physician, or the institute to which the patient has been transferred, should be contacted, or, if available, national registries consulted to obtain information on the patient being alive and to collect information on RRT history since hospital discharge. The attempts need to be documented in the patient's medical records.

12.7 End of trial and early termination

The EoT visit will be performed at Day 61 (+10 days) including the assessments as outlined in Table 1.

If a patient prematurely discontinues the trial after randomization (for reasons see Section 18), the patient will be asked to complete an early termination visit at which all assessments normally performed at Day 61 (Table 1) will be completed.

If a patient discontinues the treatment after the first IP dose, an early termination visit (see Table 1) will be performed including safety laboratory and a pregnancy test, if applicable. If possible, the patient should also return to the site on the actual Day 61 for an additional sCreat sample.

In case of early termination, reasons, circumstances, and findings should be fully described on the Early Termination page in the eCRF respecting the patient's rights.

12.8 Medical care upon termination of the clinical trial

After termination of the trial, patients will not receive any further trial-specific treatment (trial-specific treatment ends at the treatment visit). Patients who discontinue the trial or IP treatment, prematurely or as scheduled, will be treated according to the investigator's discretion and standard treatment guidelines, irrespective of the reason for discontinuation.

12.9 Restrictions and contingency measures due to serious public health emergencies

If patients are unable to attend scheduled on-site visits for their own safety or the safety of others, or due to restrictions on leaving home, or restrictions on social contacts, the visits may be performed by telephone.

During the telephone visits, at least the following will be assessed:

- Check if the patient is still alive
- AEs
- Changes in concomitant medications taken by the patient
- ICU stay (admission and discharge date and reason)
- RRT history (including intermittent RRT) since hospital discharge
- Complications included in the STS definition of surgical complications

Also, trial visits may be carried out as home visits, if possible and desired by the patient. The patient's consent to these visits will be covered in the ICF. Home visits may only be performed by trial personnel or by a third vendor, if allowed by local regulations. The sponsor will decide on a country-specific basis if and to which extent any organizational measures as described above will be performed to mitigate restrictions during public health emergencies.

All general and site-specific rules for performing the required examinations must be considered. If patients withdraw their consent for participation in the trial, the investigator will contact the patients by telephone to assess their health condition.

13 Efficacy assessments

13.1 Serum creatinine level

The sCreat values will be measured at Screening (if applicable, existing values not older than 28 days can be used), at Baseline, at Day 1 post-surgery, daily through Day 5, and on Day 61. The values at post-surgery Day 1 and Days 2, 3, 4, and 5 compared to the value at Baseline will be used to calculate the sCreat ratio. sCreat will be collected immediately after surgery before the second dose of IP when the patient is disconnected of the CPB, and thereafter in 24-hour intervals after the start of surgery (± 2 -hour time window).

13.2 MAKE 60

MAKE 60 is defined as death or having received or are receiving new RRT or having a decrease in eGFR $\geq 25\%$ compared to Baseline (calculated with sCreat) at Day 61 within the trial after surgery.

Date and cause of death up to Day 61 will be recorded on a separate page in the eCRF. In addition, the underlying cause of death must be reported as an SAE. The all-cause mortality at Day 61 will be used for the calculation of MAKE 60. If a patient is not able to return for Day 61

visit, reasonable efforts should be taken to contact the patient, patient's legal representative, patient's primary care physician, or the institute to which the patient has been transferred, to obtain information on the patient being alive, or by consulting national registries if available.

The CKD-EPI formula using sCreat [1] will be used for the eGFR calculation without correction for race.

All modalities of RRT are allowed in the trial, including continuous, intermittent, and other non-continuous RRT modalities. New onset RRT is defined as any form of RRT (temporarily or permanent) applied during the period between Surgery and Day 61. Any RRT observed up to and including Day 61 will be used for the calculation of MAKE 60.

Initiation of RRT should preferably be based on the below criteria as described by Schneider et al [58]. A patient who meets at least one of the below criteria will be eligible for RRT initiation:

- Anuria (negligible urine output for 6 hours)
- Severe oliguria (urine output <200 mL over 12 hours)
- Hyperkalemia (potassium concentrations >6.5 mmol/L)
- Severe metabolic acidosis (pH <7.2 despite normal or low partial pressure of carbon dioxide in arterial blood)
- Volume overload (especially pulmonary edema unresponsive to diuretics).
- Pronounced azotemia (urea concentrations >30 mmol/L or creatinine concentrations 300 µmol/L)
- Clinical complications of uremia (e.g., encephalopathy, pericarditis, neuropathy)

Investigators may use other criteria based on clinical judgment and standard practice. Regardless, the main reason for RRT initiation will be recorded in the eCRF, as well as the modality and the start and stop dates of RRT. Modalities other than continuous RRT are defined for purposes of this protocol as intermittent RRT.

13.3 KDIGO AKI stage

The KDIGO AKI stage will be assessed after surgery daily through Day 5 using sCreat values. The staging (modified from KDIGO Clinical Practice Guideline for AKI [57]) used for AKI severity is defined in **Error! Reference source not found..**

Table 4: Staging of acute kidney injury

Stage	Serum creatinine
0	<1.5 times the baseline value
1	1.5 to 1.9 times baseline value OR ≥0.3 mg/dL (≥26.5 µmol/L) increase
2	2.0 – 2.9 times baseline value
3	3.0 times baseline value OR increase to ≥4.0 mg/dL (≥353.6 µmol/L) OR initiation of RRT

13.4 Length of intensive care unit stay after surgery

The admission and discharge date at the ICU for the planned cardiac surgery will be recorded in the eCRF. For all-cause ICU stay during the trial, the start and end date and the reason will be collected up to visit Day 28.

13.5 Biomarkers

Blood (serum and plasma) and urine samples for potential future biomarker determinations by a laboratory will be collected at Baseline, Day 1 post-surgery (specification for blood sampling: after disconnection of the CPB but before start of second IP dose), Day 2, Day 3, Day 4, Day 5, and Day 61 (only for blood sampling). The blood sampling for biomarkers should be done at the same time as for sCreat, within a time window of ±1 hour to the sampling for sCreat.

Blood sampling for biomarkers consists of a serum sample for future sCreat and cystatin C determination, a plasma EDTA sample and a plasma citrate sample for potential future biomarker analysis.

Spot urine is collected and will be analyzed for biomarkers in the future. Processing of the urine samples is described in detail in the laboratory manual. The collection date and start and stop time of urine sampling must be recorded in the eCRF together with the volume of urine collected.

The blood and urine samples will be stored up to five years after publication of the trial results in a clinical trial report for the determination of biomarkers, including new biomarkers of RD which may become available (see the laboratory manual for further information).

14 Pharmacokinetic assessments

Blood samples will be collected for determination of ilofotase alfa concentration on Day 1 post-surgery (after disconnection of the CPB, but prior to second dose of IP), Day 2, Day 3, Day 4, and Day 5. Time and date of sample collection must be recorded in the eCRF.

Confidential

Blood samples will be collected, handled, labelled, stored, and shipped according to the laboratory manual for central analysis.

A model based (compartmental) evaluation is foreseen. Data will be added to an existing PopPK model and fitted to evaluate at least clearance and volume of distribution in this population. The influence of relevant covariates might be explored as well as a comparison with previous observations captured in the PopPK model.

15 Safety assessments

15.1 Adverse events

15.1.1 Definitions

An **AE** is any untoward medical occurrence in a patient or clinical investigation patient administered a medicinal product and which does not necessarily have to have a causal relationship with the treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

Any worsening in severity or frequency of a concomitant disease or any new disease diagnosed in the trial must be documented as an AE.

Clinically significant abnormal laboratory findings related to progression of the underlying disease and cardiac surgery (unless judged by the investigator to be more severe than expected for the patient's condition) are not considered AEs. In addition, abnormal laboratory findings may not be considered AEs if there is no change compared to baseline values.

The disease/disorder being studied or expected progression, signs, or symptoms of the disease/disorder being studied, unless more severe than expected for the patient's condition will not be considered as (S)AEs.

The clinical outcomes expected during the post-operative course (i.e., common surgical complications) will not be considered as (S)AEs, unless the investigator considers related to the medicinal product. Common surgical complications considered not related to the medicinal product will be documented in general terms using a standardized form in the eCRF.

A surgery or procedure scheduled to occur during the trial will not be considered an AE if the surgery or procedure will be performed for a pre-existing condition and the surgery or procedure was planned prior to trial entry. However, if the pre-existing condition deteriorates unexpectedly during the trial (e.g., surgery performed earlier than planned), then the

deterioration of the condition for which the surgery or procedure is being done will be considered an AE.

All AEs which occur after start of the first administration of the IP up to 14 days after last IP exposure are defined as **TEAEs**.

A **SAE** is any AE occurring at any dose that:

- results in death
- is life-threatening
- requires inpatient hospitalization or prolongation of existing hospitalization
- results in persistent or significant disability or incapacity
- results in a congenital anomaly or birth defect
- is an important medical event

Important medical events that may not have resulted in death, were not life-threatening, or did not require hospitalization are considered an SAE when, based upon appropriate medical judgment, they may have jeopardized the patient and required medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization; or development of drug dependency or drug abuse.

An AE is considered life-threatening if, in the view of either the investigator or sponsor, its occurrence places the patient at immediate risk of death. It does not include an AE that, had it occurred in a more severe form, might have caused death.

An **adverse drug reaction (ADR)** is any noxious and unintended response to a medicinal product related to any dose of the product. In accordance with ICH guideline-E2A, the definition of an adverse reaction implies a reasonable possibility of a causal relationship between the AE and the IP.

An **unexpected ADR** is an adverse reaction, the nature, severity, or outcome of which is not consistent with the applicable reference document (reference safety information in the investigator's brochure). Reports which add significant information on specificity or severity of a known, already documented AE constitute unexpected AEs, too. For example, an event more specific or more severe than described in the reference document would be considered unexpected.

A **SUSAR** is a suspected adverse reaction related to the IP (i.e., possibly, probably, and definitely related) that is both unexpected and serious. SUSARs will be subject to expedited regulatory reporting.

15.1.2 Classification of adverse events

Causality

The causal relationship between the AE and the IP and trial procedure will be assessed as follows:

- | | |
|---------------------|--|
| Definitely related: | There is a reasonable temporal relationship between the trial intervention (e.g., IP and/or procedure) and the AE, and no other obvious explanation for the AE exists. |
| Probably related: | There is a reasonable temporal relationship between the trial intervention (e.g., IP and/or procedure) and the AE, based upon the known pharmacological action of the IP, known or previously reported adverse reactions to the IP or class of drugs, or judgment based on the investigator's clinical experience, the association of the event with the IP seems likely. The event disappears or decreases on cessation or reduction of the dose of IP. |
| Possibly related: | There is a reasonable temporal relationship between the trial intervention (e.g., IP and/or procedure) and the AE, and the AE could also be explained by disease, other medications, or another obvious explanation for the AE exists. |
| Unrelated: | The AE is independent of any trial intervention (e.g., IP and/or procedure), or evidence exists that the event is definitely related to another etiology. |

Severity

Severity is a clinical observation and describes the intensity of the event.

- | | |
|-----------|---|
| Mild: | Transient symptoms, no interference with the patient's daily activities. |
| Moderate: | Marked symptoms, moderate interference with the patient's daily activities. |
| Severe: | Considerable interference with the patient's daily activities. |

Outcome Categories

Resolved:	The patient has fully recovered from the event, or the condition has returned to the level observed at Baseline.
Resolving:	The patient has recovered from the event, but the condition has not returned to the level observed at Baseline.
Not resolved/ Not recovered:	The event is ongoing at the time of reporting and the condition has not returned to the level observed at Baseline.
Resolved with sequelae:	As a result of the AE, the patient suffered persistent and significant disability/incapacity (e.g., became blind, deaf, or paralyzed) or the condition became chronic or stabilized and no worsening is expected.
Death:	The patient died due to the event. If the patient died due to other circumstances than the event, the outcome should be stated otherwise (e.g., not resolved or resolving).
Unknown:	If outcome is not known or not reported.

Action taken with IP

The action taken with the IP will be assigned to one of the following categories:

Dose not changed:	No change in the dose of the IP.
Drug withdrawn:	Discontinuation of the IP.
Unknown:	The information is unknown or implausible and it cannot be supplemented or verified.
Not applicable:	This category should be used in circumstances such as when the patient has died, or the treatment had been completed prior to reaction(s) or event(s) or the IP had not been administered.

15.1.3 Documentation of adverse events

All AEs that occur after the patient has signed the ICF up to and including Day 28 or patient's trial completion, whichever occurs first, must be recorded. After Day 28, until Day 61 or patient's trial completion, only AEs that meet the SAE criteria and that the investigator considers possibly or definitely related to the IP should be reported to the sponsor or designee. The occurrence of AEs should be sought by non-directive questioning of the patient at each visit during the trial, e.g., "Have you experienced any problems since your last contact?" AEs

also may be detected when they are volunteered by the patient during or between visits or through physical examination, laboratory test, or other assessments.

The AE term should be reported in standard medical terminology, if possible.

All AEs which occur during the trial will be recorded in the patient's AE section of the eCRF and will include the following information: a description, date of onset and resolution, severity, seriousness, relationship to IP (you may distinguish between tested drug and comparator), relationship to study procedure, action taken and outcome. For SAEs, the SAE Form must also be completed (see Section 15.1.4).

If possible, a diagnosis should preferably be entered as the AE term rather than signs or symptoms of an event. If a diagnosis changes over time, the event term should be modified accordingly.

Changes in severity or frequency: A continuous event changing in severity will be considered one event with the most severe intensity documented.

Intermittent AEs will only be recorded once, as long as the severity does not change and 'intermittent' is added to the verbatim text of the AE term. If, however, the AE changes from intermittent to continual, the original event should be closed out and reopened as a new AE.

Changes in seriousness: A continuous event with a changing seriousness will be considered as one event, but the start and stop date of the time the event is serious must be separately documented.

15.1.4 Reporting of serious adverse events and suspected unexpected serious adverse reactions

The investigator must record all SAEs on the SAE report form within the eCRF immediately, without undue delay but not later than within 24 hours of obtaining knowledge.

The initial report should contain as much information as possible, but at least the following information:

- Patient identification code
- Protocol number and title
- Event term (only one term should be entered)
- Name of the investigator
- Causality assessment (relationship to IP or study procedure)

All SAEs must be followed by the investigator until the event has resolved or resolved with sequelae or the patient died, whichever occurs first. The investigator should complete missing

or requested information and submit follow-up reports, as appropriate. Some events do not have an “end”, such as metastasis; however, once these events are determined by the investigator to be stable or chronic, the investigator may consider the event to be resolved or resolved with sequelae.

SUSARs will be expedited to independent ethics committees (IECs), competent authority (CA), and investigator following pertinent national legislation.

Within the EU all relevant information about any SUSAR will be reported electronically and without delay to the EudraVigilance database. The reporting period for SUSARs is based on the severity of the reaction and is determined as follows:

- fatal or life-threatening SUSARs, as soon as possible and in any event not later than 7 days after the sponsor became aware of the reaction
- non-fatal or non-life-threatening SUSARs, not later than 15 days after the sponsor became aware of the reaction
- SUSARs which were initially considered to be non-fatal or non-life-threatening, but which turn out to be fatal or life-threatening, as soon as possible and in any event not later than 7 days after the sponsor became aware of the reaction being fatal or life-threatening.

Where necessary to ensure timely reporting, the sponsor may submit an initial incomplete report followed up by a complete report.

If investigators become aware of an SAE with a suspected causal relationship to the IP occurring after the end of the clinical trial in a patient treated by them, investigators shall, without undue delay, report the SAE to the sponsor.

15.1.5 Follow-up of adverse events

All SAEs judged to be related to the IP must be followed by the investigator until the patient has recovered, recovered with sequelae, died, or until the investigator determines that the patient’s condition is stable, whichever occurs first. All other AEs must be followed by the investigator until the conditions mentioned above are met or until the Day 28, whichever comes first, and until all AE-related queries for the patient have been resolved.

The investigator will take all appropriate and necessary therapeutic measures required for resolution of the AE, if applicable. All efforts to collect follow-up information must be documented in the source data.

Follow-up information should be supplied on the respective forms of the eCRF.

15.2 Pregnancy

Any pregnancy that occurs during trial participation in any female patient or female partner of a male patient must be recorded on the appropriate pregnancy form and reported within 24 hours of the first awareness of the event to the sponsor or its designee.

Patients who become pregnant during the trial after IP administration should stay in the trial.

If a pregnancy is demonstrated/observed after screening or randomization but before IP administration, administration of IP should not be initiated.

Pregnancy is not regarded as an AE unless there is a suspicion that the IP may have interfered with the effectiveness of a contraceptive method.

The outcome of all pregnancies (spontaneous or induced abortion, normal or complicated delivery, stillbirth, and normal offspring or congenital abnormality) and the status of mother and child, even if the patient was discontinued from the trial, must be followed up and documented. All reports of spontaneous abortion, ectopic pregnancy, stillbirth, neonatal death, or congenital anomaly are SAEs. Induced abortions without complications should not be handled as AEs. Hospitalization for normal delivery of a healthy newborn should not be considered an SAE.

Pregnancy follow-up will be recorded on the pregnancy outcome form and will include an assessment of the possible relationship of the IP to any pregnancy outcome. The pregnancy outcome form should be reported by the investigator within 30 days after delivery knowledge to the sponsor or its designee.

15.3 Medication error and overdose

Medication errors, overdose, abuse, and misuse must be documented on the respective eCRF page and are defined as follows:

- **Medication error:** Any unintentional error in the administration of the IP while in the control of the healthcare professional. Examples for medication errors include administration of expired IP, administration of IP that has undergone temperature excursion from the specified storage range, use of IP outside of what is foreseen in the protocol, administration of IP to subjects not involved in the trial or foreseen to receive another IP.
- **Overdose:** Administration of a quantity of the IP given per administration or cumulatively which is above the maximum dose (see Section [11.1.5](#)).

Stop and start date as well as the action with IP due to medication errors or overdose must be documented as follows:

- No changes
- IP temporarily interrupted
- Dose adjusted
- Other

If a medication error or overdose is accompanied by an AE, as determined by the investigator, the AE should be recorded on the AE page of the eCRF.

15.4 Laboratory investigations

Blood samples for clinical safety laboratory tests including sCreat will be collected at the times indicated in Table 1 and analyzed by local laboratory. Details about the handling and labeling of biological samples are provided in the laboratory manual. The samples will be destroyed after completion of the analyses for this trial.

All safety and screening laboratory assessments and parameters are specified in Table 5.

Note: AP activity measurement and/or reporting is NOT allowed from the first dosing with the IP up to and including Day 14. If for example, automated AP activity measurements cannot be avoided, the results from blood samples taken during the first 14 days of the trial could lead to erroneous interpretation of liver function, as ilofotase alfa administration will increase the measured AP activity. AP activity results in this period are then not to be reported to trial team members or to any other blinded personnel involved in patient care and/or data collection as this could lead to unblinding (see Section 10.3).

The laboratory reports received from the laboratory will be assessed by the investigator. Clinically significant abnormal laboratory findings as judged by the investigator should be recorded as AE in the eCRF as described in Section 15.1.1.

Table 5: Clinical safety and screening laboratory parameters

Laboratory assessment	Parameters
Hematology	red blood cell count, hemoglobin, hematocrit, platelet count, white blood cell count with differentials (absolute counts of neutrophils, lymphocytes, monocytes, eosinophils, basophils)
Clinical chemistry	Liver: aspartate aminotransferase, alanine aminotransferase, gamma-glutamyl transferase, total bilirubin Kidney: blood urea nitrogen or urea, creatinine Other: potassium, chloride, bicarbonate, sodium, total protein, albumin, glucose

A serum or urine human chorionic gonadotropin pregnancy test will be performed for all women of childbearing potential at the times indicated in [Table 1](#). The pregnancy test will be performed at the local hospital laboratory. A woman is considered of childbearing potential, i.e., fertile following menarche and until becoming post-menopausal unless permanently sterile. Permanent sterilization methods include hysterectomy, bilateral salpingectomy and bilateral oophorectomy. A postmenopausal state is defined as no menses for 12 consecutive months without an alternative medical cause with confirmatory FSH level of ≥ 40 mIU/mL. The FSH level is determined at Screening in post-menopausal women of age ≤ 60 years.

15.5 Anti-drug antibodies

Blood samples will be taken for ADA determination at Baseline and on Day 61 or EoT in case of prematurely discontinuation after IP administration. The date and time of blood collection will be documented in the eCRF.

Immunological testing will be performed by a central laboratory by using an enzyme linked immunosorbent assay on a Meso Scale Discovery MSD platform. The assay is validated in human serum samples.

15.6 Physical examination and electrocardiogram

Physical examination

The physical examination will include examination of the head, neck, eyes, ears, nose, and throat, cardiovascular system, pulmonary system, lymphatic system, thyroid gland, muscular and skeletal system, general appearance, extremities, abdomen, skin (whole body), and neurological system and will be performed at Screening only. Height and weight measurement to calculate the body mass index of the patient will be performed at Baseline.

If the physical examination, height and weight measurements are done as standard of care on admission of the patient to the hospital, they do not need to be repeated at Baseline, if they were done maximally within 72 hours pre-surgery. These data will be used for baseline assessment.

Electrocardiogram

A 12-lead ECG obtained prior to start of surgery (Baseline ECG) must be captured in source documents. ECGs obtained for clinical purposes within 48 hours of starting the surgery may be used as Baseline ECG, provided there has been no clinically significant change in the cardiac status between the time of its recording and the start of the surgery. The purpose of the Baseline ECG is to have a status at entry to which later ECGs may be compared, should this become relevant.

16 Other assessments

16.1 EuroScore II assessment

The score will be assessed at Baseline and calculated according to the risk calculator (<http://www.euroscore.org/calc.html>) [59]. Definitions and explanations of the risk factors can be found below.

- NYHA class. NYHA classification for dyspnea:
 - I: no symptoms on moderate exertion
 - II: symptoms on moderate exertion
 - III: symptoms on light exertion
 - IV: symptoms at rest
- Canadian Cardiovascular Society grading 4 angina (inability to perform any activity without angina or angina at rest)
- Insulin-dependent diabetes mellitus
- Extracardiac arteriopathy. One or more of the following:
 - claudication
 - carotid occlusion or >50% stenosis (North American Symptomatic Carotid Endarterectomy Trial criteria)
 - amputation for arterial disease
- Previous or planned intervention on the abdominal aorta, limb arteries or carotids
- Poor mobility. Severe impairment of mobility secondary to musculoskeletal or neurological dysfunction
- Previous cardiac surgery. One or more previous major cardiac operation involving opening the pericardium
- Renal dysfunction. This is assessed by Creat clearance as estimated using the Cockcroft–Gault formula and falls into 3 categories:
 - Creat clearance 51–85
 - on dialysis (regardless of sCreat)
 - Creat clearance ≤ 50
- Active endocarditis. Patients still on antibiotic treatment for endocarditis at the time of surgery
- Critical preoperative state. Any one or more of the following occurring preoperatively in the same hospital admission as the operation:
 - ventricular tachycardia or fibrillation or aborted sudden death
 - cardiac massage
 - ventilation before arrival in the anesthetic room
 - inotropes

- intra-aortic balloon counter pulsation or ventricular-assist device before arrival in the anesthetic room
- Acute renal failure (anuria or oliguria <10 mL/h)
- LV function or LVEF:
 - good (LVEF 51% or more)
 - moderate (LVEF 31–50%)
 - poor (LVEF 21–30%)
 - very poor (LVEF 20% or less)
- Urgency of procedure:
 - elective: routine admission for operation
 - urgent: patients not electively admitted for operation but who require surgery on the current admission for medical reasons and cannot be discharged without a definitive procedure
 - emergency: operation before the beginning of the next working day after decision to operate
 - salvage: patients requiring cardiopulmonary resuscitation (external cardiac massage) en route to the operating theatre or before induction of anesthesia. This does not include cardiopulmonary resuscitation after induction of anesthesia
- Recent MI. Within 90 days before operation
- Weight of procedure. This measures the extent or size of the intervention. The Baseline is isolated CABG: operations ‘heavier’ than the Baseline are in 3 categories:
 - isolated non-CABG major procedure (e.g., single valve procedure, replacement of ascending aorta, correction of septal defect, etc.)
 - 2 major procedures (e.g., CABG + AVR), or CABG + mitral valve repair (MVR), or AVR + replacement of ascending aorta, or CABG + maze procedure, or AVR + MVR, etc.)
 - 3 major procedures or more (e.g., AVR + MVR + CABG, or MVR + CABG + tricuspid annuloplasty, etc.), or aortic root replacement when it includes AVR or repair + coronary reimplantation + root and ascending replacement)

Only major cardiac procedures count towards the total. Examples of procedures which do not qualify are sternotomy, closure of sternum, myocardial biopsy, insertion of intra-aortic balloon, pacing wires, closure of aortotomy, closure of atriotomy; removal of atrial appendage, coronary endarterectomy as part of CABG, etc.

16.2 Peri-surgical factors and surgery related complications

For the documentation of surgery related complications, the Society of Thoracic Surgeons (STS) national database initiative for adult cardiac surgery will be used as reference, this was

Confidential

established in 1989 as an initiative for quality improvement and patient safety in cardiothoracic surgery (<https://www.sts.org>).

The following definitions will be used (<https://www.sts.org/quality-safety/performance-measures/descriptions>):

- **Operative mortality**
Number of patients who die, including both 1) all deaths occurring during the hospitalization in which the operation was performed, even if after 30 days, and 2) those deaths occurring after discharge from the hospital, but within 30 days of the procedure during hospitalization regardless of length of stay or within 30 days of surgery if discharged
- **Surgical re-exploration**
Number of patients who require return to the operating room for mediastinal bleeding with or without tamponade, graft occlusion, valve dysfunction, or other cardiac reason during the hospitalization for surgery, which includes the entire postoperative period up to discharge, even if over 30 days
- **Postoperative renal failure**
Number of patients who develop postoperative renal failure or require dialysis during the hospitalization for surgery, which includes the entire postoperative period up to discharge, even if over 30 days. Renal failure is defined as an increase in sCreat levels 4 mg/dL or greater (176.8 mmol/L), a 50% or greater increase in sCreat levels over the Baseline preoperative value, or a new requirement for dialysis
- **Deep sternal wound infection**
Number of patients who, within 30 days postoperatively, develop deep sternal wound infection involving muscle, bone, and/or mediastinum requiring operative intervention. Must have all of the following conditions within 30 days postoperatively:
 - wound opened with excision of tissue (I&D) or re-exploration of mediastinum
 - positive culture unless patient on antibiotics at time of culture or no culture obtained
 - treatment with antibiotics beyond perioperative prophylaxis
- **Postoperative prolonged intubation (ventilation)**
Number of patients who require intubation >24 hours during the hospitalization for surgery, which includes the entire postoperative period up to discharge, even if over 30 days
- **Stroke/cerebrovascular accident**
Number of patients who have a postoperative stroke (i.e., any confirmed neurological deficit of abrupt onset caused by a disturbance in blood supply to the brain) that did not resolve within 24 hours during the hospitalization for surgery, which includes the entire postoperative period up to discharge, even if over 30 days

Confidential

- **Myocardial infarction**

Number of patients who have a postoperative myocardial infarction during the hospitalization for surgery, which includes the entire postoperative period up to discharge, even if over 30 days

The surgical parameters, type of surgery actually performed, number of valves and types of valves, number of bypasses, surgical approach, duration of surgery (time between first incision and last skin suture), type and volume of cardioplegia, priming volume CPB pump, time on CPB pump, cross-clamp time, lowest hematocrit recorded in the operating room, estimated blood loss (if applicable), packed cells needed, time and number of episodes with mean blood pressure <50 mmHg during surgery will be recorded on Day 1.

The use of vasopressors and/or inotropic drugs will be recorded on a daily basis from Day 1 through Day 5.

For inotropic and vasopressor agents, the generic name, start and stop date and time and the maximum dose will be recorded in the eCRF.

17 Statistical methods

Statistical methods are briefly described below and will be further detailed in a statistical analysis plan (SAP), which will be finalized before database lock.

17.1 Sample size calculation

The effect size of 20% has been estimated based on the evaluation of data from patients with complex cardiac surgery and pre-operative impaired renal function from the CERNER database (AM-Pharma report RAP-CLIN-0159). Assuming a mean of 1.28 sCreat ratio for the placebo arm, a pooled standard deviation of 0.465 and a one sided alpha of 2.5%, a 20% difference in sCreat ratio between treated and control patients can be detected with a power of 91.7% and a sample size of 150.

To detect a 15% difference in sCreat ratio between treated and control patients up to 250 patients are needed with a similar power (90.1%) and equal assumption on mean, SD and alpha.

Drop-outs are not considered within the 250 patients.

17.2 General approach

Unless otherwise stated, continuous data will be summarized descriptively, i.e. number of patients, mean, standard deviation, median, quartiles and range (minimum and maximum).

Categorical variables will be summarized by absolute and relative frequencies (percentages) of patients by category.

17.3 Analysis sets and types of analyses

Screened set

The screened set will consist of all patients who provided informed consent.

Safety analysis set

The safety analysis set (SAF) will consist of all patients who received at least one dose of IP. If it is uncertain if the patient has received IP, the patient will be included in the SAF. The assignment of patients to the treatment groups will be as actually treated. The SAF will be the analysis set for all safety analyses including secondary endpoint.

Full analysis set

The full analysis set (FAS) will consist of all patients who were randomized and received both planned doses of IP. In accordance with the intent-to-treat-principle, the assignment of patients to the treatment groups will be as randomized. The FAS will be the analysis set applied to the evaluation of the efficacy endpoints.

Per-protocol set

The per-protocol set (PPS) will consist of all patients included in the FAS without any major PD. PDs will be identified and classified for each patient during a blind data review meeting. The assignment of patients to the treatment groups will be as randomized. The PPS will be used for supplementary analyses of the primary efficacy endpoint.

Patients will be allocated to analysis sets (SAF, FAS and PPS) during a blind data review meeting before data base lock and unblinding.

Pharmacokinetic set

The pharmacokinetic set (PKS) will include all patients who have not had any AEs or PDs deemed to impact the PK, and have sufficient quantifiable samples collected for estimation of PK parameters. More details on the PK population and on the planned analysis methods will be provided in a separate PK SAP and will not be part of the clinical trial report.

17.4 Handling of missing, unused, and spurious data

For the primary analysis, the ratio between the highest sCreat value post-surgery (post-surgery Day 1 and Days 2, 3, 4, and 5) and the sCreat value at Baseline will be derived (post-surgery/Baseline). If the baseline measurement and at least one post-surgery measurement are available and the patient is still alive after Day 5, these values will be used to calculate the ratio.

If the sCreat value at Baseline is not available, the sCreat value at Screening can be used instead. If the patient dies after the second IP application and before Day 5 the ratio will be set to 1.25 irrespectively of any measured sCreat value (see [Table 2](#)).

Further details on imputation strategies and the handling of missing data and potential supplementary analyses of the efficacy endpoints will be specified in the SAP.

17.5 Analysis of trial conduct and patient disposition

Descriptive analysis of patients in each analysis set (SAF, FAS, and PPS) will be presented overall and by treatment.

The proportions of patients who completed the trial and prematurely discontinued treatment or the clinical trial will be summarized as well as the reason for the premature termination.

17.6 Baseline characteristics

Demographic and baseline characteristics (e.g., sex, age, race, body weight, body height), EuroScore II, and 12-lead ECG will be summarized descriptively by treatment group to describe the trial population.

Medical history, physical examination and pregnancy tests will be listed. Medical history will be coded according to the MedDRA.

Concomitant medications will be coded according to Anatomical Therapeutic Chemical Classification System and number and frequency of patients with prior or concomitant medication will be summarized by treatment group. In addition, medications which are known to cause kidney problems will be tabulated separately. Relevant medications will be specified during blinded data review meeting.

17.7 Efficacy analyses

17.7.1 Primary efficacy endpoint

The primary objective is to assess the effect of ilofotase alfa on renal function. Therefore, the ratio between the highest sCreat value post-surgery (post-surgery Day 1 and Days 2, 3, 4, and 5) and the pre-surgery baseline value for sCreat will be analyzed by means of an analysis of covariance (ANCOVA). The ratio will be derived as maximum post-surgery value/ pre-surgery baseline value.

The ANCOVA methodology will test the null hypothesis that the mean sCreat ratio is equal between the two treatment groups. The baseline sCreat value will be included as covariate. To account for the stratification factors, indicator variables, ie, baseline eGFR ≤ 45 mL/min/m²

(yes/no) and type of surgery (CABG with 3 or more distal anastomoses [yes/no]), will additionally be included as covariates. In case of violations of the assumption of normality for the sCreat ratio, a sensitivity analysis will be undertaken based on the log or some other suitable transformation.

The primary analysis will be based on the FAS. The analysis will be repeated on the PPS as supplementary analysis.

Additional sensitivity analyses are planned. One analysis will further include pump time and cross-clamp time as covariates into the model. A second analysis will exclude patients 'off-pump' (no start and stop time of CPB pump) from the analysis. Furthermore, a sensitivity analysis will be done with sCreat ratio ≤ 1.25 as threshold.

If the distribution of medications which are known to cause kidney problems differ thoroughly between treatment groups the primary endpoint will be analyzed including the medications as covariate as post-hoc analysis.

17.7.2 Secondary endpoints

17.7.2.1 Efficacy

To assess the effect of treatment with ilofotase alfa on the clinical outcome in patients undergoing cardiac surgery, the occurrence of the composite MAKE endpoint will be analyzed. The components of MAKE are:

- death up to and including Day 61,
- received or are receiving new RRT up to and including Day 61, or
- a decrease in eGFR $\geq 25\%$ on Day 61 compared to the pre-surgery baseline eGFR reference value.

The logistic regression methodology will test the null hypothesis that the incidence of MAKE is equal between the two treatment groups. The pre-operative baseline sCreat value will be included as covariate. To account for the second stratification factors, type of surgery (CABG with 3 or more distal anastomoses [yes/no]), will additionally be included as covariate.

The primary and secondary efficacy endpoint will be tested in hierarchical order.

As additional sensitivity analyses, the MAKE endpoint will be analyzed without the death component and a WinRatio analysis will be performed.

The secondary efficacy analysis will be based on the FAS.

17.7.2.2 Safety

For the secondary endpoint, incidence rate of AEs and SAEs through Day 28, the absolute and relative number of patients reporting each AE (by treatment group) and severity will be evaluated to assess the safety and tolerability of ilofotase alfa in patients undergoing open chest heart surgery. SAF will be used as analysis set. For more details see Section [17.9.1](#).

17.7.3 Exploratory endpoints

To investigate incidence, duration, and severity of AKI, the AUC of AKI (KDIGO AKI stage) will be analyzed. The change in severity of AKI (KDIGO stage) over time (Day 1 post-surgery through Day 5), expressed as $AUC_{1\text{post-5days}}$ will be summarized by treatment. Severity stages of AKI are based on the change in sCreat from Baseline. The $AUC_{1\text{post-5days}}$ will be calculated using linear trapezoidal rule and will be summarized using descriptive statistics.

The overall incidence of AKI (AKI criterion is fulfilled for at least one timepoint, Day 1 to Day 5) will be analysed as well.

To assess the effect on surgery related complications the following parameters according to Section [16.2](#) will be summarized by treatment group:

- Operative mortality
- Surgical re-exploration
- Postoperative renal failure
- Deep sternal wound infection
- Postoperative prolonged intubation (ventilation)
- Stroke/cerebrovascular accident
- Myocardial infarction
- Type of surgery actually performed
- Number of valves
- Types of valves
- Number of bypasses
- Surgical approach
- Duration of surgery (time between first incision and last skin suture)
- Type of cardioplegia
- Volume of cardioplegia
- Priming volume CPB pump
- Time on CPB pump
- Cross-clamp time
- Lowest hematocrit recorded in the operating room
- Estimated blood loss

- Packed cells needed
- Time of episodes with mean blood pressure <50 mmHg during surgery
- Number of episodes with mean blood pressure <50 mmHg during surgery
- Use of vasopressors and/or inotropic drugs

To assess the duration of critical care after the scheduled surgery, length of stay in the intensive care unit after the initial surgery will be assessed. Length of stay will be measured in days and summarized using descriptive statistics.

All exploratory endpoints will be performed for the FAS.

17.8 Pharmacokinetic

PK analyses and PK population will not be included in the clinical trial report. These analyses will be done by the sponsor or delegate and will be specified in an additional SAP.

PK analyses will be performed for the PKs.

17.9 Safety analyses

All safety analyses will be performed for the SAF, if not stated otherwise.

Details of further analyses will be specified in the SAP.

17.9.1 Adverse events

AEs will be coded according to the MedDRA. All AEs noted during the trial will be listed. The absolute and relative number of patients reporting each AE (by treatment group) and severity will also be presented. The analysis will focus on the TEAEs, which will be summarized and tabulated according to primary system organ class and preferred term. TEAEs leading to death and TEAEs resulting in discontinuation of the study will be tabulated using frequency tables if a reasonable number of AEs of this type is observed.

17.9.2 Clinical laboratory

The results of the safety laboratory assessments, i.e., hematology and blood chemistry, will be listed for each patient. All laboratory variables will be assessed by the investigator with relationship to the normal range as: 'normal', 'abnormal, but without clinical relevance', or 'abnormal with clinical relevance'. Out-of-range laboratory values that are considered 'abnormal with clinical relevance' and that were not present at Screening will be documented as AEs and listed as such.

17.9.3 Anti-drug antibodies

The analysis of the ADAs will be done by ICON after the trial is completed.

17.10 Interim analysis

No interim analysis is planned for this trial.

18 Patient withdrawal from trial participation

Participation in the trial is voluntary. The patients may withdraw from treatment with the IP and/or the trial upon request at any time and for any reason without any consequences.

If a patient withdraws his/her consent for participating in this trial (i.e., the patient refuses further IP treatment and follow-up procedures), no further trial-related procedures will be performed, and no attempts will be made to collect additional data; however, data already collected up to the time of withdrawal of consent will be used. If a patient withdraws from the trial, he/she may request destruction of samples taken and not yet tested. This must be documented in the site trial records by the investigator. All patients who withdraw consent to future procedures should, if at all possible, have the EoT assessments performed immediately upon withdrawal.

Discontinuation of IP treatment

The investigator must stop administration of IP for any of the following reasons:

- Occurrence of SAE(s) which in the opinion of the investigator may jeopardize the patient's health or compromise the trial objectives

In such cases, monitoring of the patient will continue until the event has resolved or stabilized or until a determination of a cause unrelated to the IP or trial procedure is made. The specific event or laboratory finding(s) must be documented in the eCRF.

- Patient withdraws consent to IP treatment or trial participation
- Investigator or sponsor decides to discontinue the patient's participation in the trial
- Sponsor terminates the trial
- Pregnancy (see Section [15.2](#))

Patients who are discontinued from treatment with the IP for the above-mentioned reasons (either at their own request or by the investigator) should not be considered withdrawn from the trial unless they withdraw their consent. The patient should continue all planned follow-up assessments as per protocol for safety and to allow for analysis of the specified endpoints. If the surgery of a patient is postponed after the first IP dose and for longer than 24 hours, an early

termination visit ([Table 1](#)) will be performed including safety laboratory and a pregnancy test, if applicable. If possible, the patient should also return to the site on the actual Day 61 for an additional sCreat sample.

Discontinuation of trial participation

The investigator must withdraw a patient from the trial for any of the following reasons:

- Withdrawal of consent to trial participation
- Lost to follow-up
- Any condition or noncompliance which in the opinion of the investigator no longer permits safe participation in the trial
- Significant protocol violation
- Trial is terminated by the sponsor
- Death

The investigator can stop a patient's participation in the trial at any time, if continuation could lead to disadvantages for the patient which cannot be justified. For all patient withdrawals, it must be recorded if the patient is withdrawn from trial participation or IP treatment and the reason for the withdrawal.

For patients who miss trial visits without stating an intention to withdraw, the investigator should make every reasonable effort to contact the patient or his/her legal representative and document in the source documents steps taken to contact, e.g., dates of telephone calls with outcome, registered letters, etc.

If withdrawal is due to an SAE or SAEs are ongoing at the time of withdrawal, SAEs will be followed up as specified in [Section 15.1.5](#).

Replacement of patients

If a patient is withdrawn from the trial before receiving any IP, the patient will be replaced (i.e., enrollment of a further patient, which will be randomized independently from the randomization of the "to be replaced patient").

19 Ethical and legal requirements

19.1 General requirements

The trial will be performed in accordance with the protocol, the Clinical Trials Regulation (EU) No 536/2014 [\[60\]](#), ICH guideline for good clinical practice (GCP; CPMP/ICH/135/95), the

applicable laws and regulations, and the Declaration of Helsinki in its currently acknowledged version.

All parties involved in the trial (i.e., investigator, sponsor, sponsor's representative, and laboratories) must also comply with the EU General Data Protection Regulation [61] and the data protection laws of the countries in which the clinical trial is conducted. This covers national or sub-national acts on data protection as well as acts with provisions for data processing in clinical trials.

19.2 Independent ethics committee or institutional review board

Before the initiation of the clinical trial, the final protocol, any amendments if applicable, the patient information sheet and consent form, as well as any additional documents which are required by national regulations and the IEC will be submitted to the competent IEC for review.

In the EU, all documents which are required by the Clinical Trials Regulation (EU) No 536/2014, national regulations, or the national IEC will be submitted via the Clinical Trials Information System (CTIS) to each participating EU member state for authorization. Before the initiation of the clinical trial in an EU member state, the authorization of that EU member state must be obtained. The ethical review is part of that authorization process and shall be performed by an IEC in accordance with the law of the EU member state concerned.

A favorable opinion for the clinical trial must be obtained from the IEC before any patient is enrolled.

If appropriate, any additional requirements imposed by the IEC will be followed. Amendments to the trial documents must be authorized before implementation, if required.

19.3 Patient recruitment and informed consent procedure

Center and patient recruitment

Experts in the field of CSA-RD in respective hospital centers will be recruited for the trial. Each clinical center will be chosen based on their documentation for patient availability, standard of care settings, previous experience in clinical trials, resources to conduct the clinical trial, and facility equipment among other things.

Patients will be recruited from patient pools at the centers, referrals from other practices, and via online-recruitment.

Patient information and consent procedure

Before any clinical trial-related activities are performed, the investigator (or authorized designee) must review the ICF and explain the trial to potential trial patients. The investigator (or authorized designee) must ensure that the patient is fully informed about the nature,

Confidential

objectives, procedures, implications, potential risks, any inconveniences, and expected benefits of the clinical trial. It must be emphasized that participation is voluntary, and that the patient has the right to withdraw from the clinical trial at any time without prejudice. Before consenting, the patient must be left with ample time to consider and ask questions. The patient must then sign and date the consent form before the conduct of any trial procedures. The investigator (or authorized designee) must sign and date the informed consent as well.

A copy of the patient information and ICF will be given to the patients for their records. The rights and welfare of the trial patients will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this clinical trial.

If amendments to the final trial protocol affect the patient's participation in the clinical trial (e.g., a change in any procedure), the patient information and ICF must be updated to incorporate this modification, and patients must sign the amended form indicating that they re-consent to participate in the clinical trial.

19.4 Insurance coverage

Insurance coverage for injuries or damages emerging from the clinical trial will be provided according to applicable legal requirements. During the informed consent procedure, the investigator (or authorized designee) must inform the patient accordingly. Insurance details will be provided to the patient within the patient information sheet.

19.5 Submission to authorities

Documents required for the trial application will be submitted to the responsible CA. In the EU, documents required for the trial application will be submitted via CTIS to each participating EU member state for authorization. Before initiation of the clinical trial in an EU member state, the authorization of that EU member state must be obtained. A national CA is involved in this authorization process according to the law of the respective EU member state. Amendments to the trial documents, which have a substantial impact on the safety or rights of the patients or on the reliability and robustness of the data generated in the clinical trial, must be authorized by the EU member states concerned and the competent authorities of any other concerned countries before implementation, if required.

19.6 Patient confidentiality

The trial protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the clinical trial or the data will be released to any unauthorized third party without prior written approval of the sponsor.

If applicable: In the context of this clinical trial, pseudonymized data and biological samples of patients will (may) be transferred to countries outside the EU and the European Economic Area for the purpose of data analysis, marketing authorization and monitoring. These might be countries for which the European Commission has not determined an adequate legal level of data protection.

With recipients in such countries, appropriate safeguards are agreed in the form of contractual data protection clauses adopted by the European Commission, so that patients have comparable enforceable rights and effective remedies as within the EU.

However, there are also countries or recipients of trial data and biological samples with which such guarantees cannot be agreed. In these cases, there is no guarantee that the data processing can be controlled in an equivalent manner by independent authorities and courts and/or that enforceable rights and effective remedies are available to patients. Furthermore, there may be more extensive rights of access by government bodies and/or private entities, which may cause significant personal disadvantages to patients. The patient's pseudonymized data can only be transferred to these countries if patients have expressly consented to this. Patients cannot participate in this clinical trial without their explicit consent to the transfer of their data to countries without an adequacy decision and appropriate safeguards.

Personal patient data will be kept confidential in compliance with the EU General Data Protection Regulation [61] and other applicable international and national requirements. Appropriate measures will be implemented to mitigate possible adverse effects in case of a data security breach. Immediate steps to limit further access to, or distribution of the affected information and to reduce the possible compromise of other information will be taken. Measures taken depend on the data security breach identified and will include the disconnection of the system that has been breached, setting or changing passwords, turning on 2-factor authentication (if not already applicable), attempt to recall unread emails, change computer access privileges, device encryption, backup copies of data, implement or revise training practices for trial staff, and disconnect internet connectivity.

In addition, the individual affected will be promptly notified.

The investigator must ensure that the pseudonymity of trial patients will be maintained and that their identities are protected from unauthorized parties. On eCRFs, compensation documentation, or any other documents submitted to the sponsor or sponsor's representative, patients must be identified only by their patient number; it is not allowed to use their names, addresses, telephone numbers, or similar information. The investigator will keep the original of the patient identification log (including complete name and date of birth of each patient) in his/her file. The investigator must maintain these documents in strict confidence.

To allow compliance with GCP, all patients will be asked for consent regarding the access to their personal clinical trial-related data for monitoring, audits, and inspections as well as regarding transmission and storage of their pseudonymous data; a respective statement will be part of the ICF. Professionals getting access to source data for monitoring, audits and inspections are bound to preserve strict confidentiality.

20 Criteria for premature termination of the trial and for closing a trial center

20.1 Criteria for terminating the clinical trial

The sponsor reserves the right to halt or terminate the trial at any time. Reasons for termination include but are not limited to:

- Potential health risk for patients
- High withdrawal rate
- New scientific knowledge becomes available that makes the objectives of the trial no longer feasible or valid
- Insufficient enrollment of patients
- Request of the sponsor or regulatory authority, or IEC or EU member state withdraws its approval

If the trial is prematurely terminated or suspended, the sponsor will promptly notify the relevant CA, EU member states concerned via CTIS, IEC, and the investigator(s) in writing about the termination of the trial and its reasons.

20.2 Criteria for closing a trial center

A trial center may be closed for the following reasons:

- The center is unable to recruit enough patients within the agreed time frame
- The center does not respond to trial management requests
- Repeated protocol or GCP violations

The investigator may terminate trial participation prematurely. In this case, the investigator will notify the sponsor in writing stating the reasons for early termination. In terminating the trial, the sponsor and the investigator will ensure that adequate measures are taken to protect the patients' interests. The investigator will return all IP supplies and all related trial material, as applicable, to the sponsor.

Confidential

The relevant CA and/or IEC will be notified (if applicable via CTIS).

21 Trial protocol, documentation and archiving of data

21.1 Modifications to the protocol

Any change to the protocol concerning e.g., the purpose of the trial, the trial design, or the patient's eligibility can only be made in the form of a written amendment to the trial protocol. Protocol waivers are not allowed. These modifications must be discussed and signed by all relevant parties before implementation.

A substantial modification is any change to any aspect of the clinical trial which is made after notification of a decision referred to in Articles 8, 14, 19, 20 or 23 of the Clinical Trials Regulation (EU) No 536/2014 and which is likely to have a substantial impact on the safety or rights of the patients or on the reliability and robustness of the data generated in the clinical trial. Substantial modifications will be submitted to the CA and IEC for an authorization and favorable opinion as required by applicable regulations (via CTIS to each participating EU member in the EU).

If these modifications affect the patients' participation in the clinical trial (e.g., a change in any procedure), the patient information sheet and ICF must be updated to incorporate this modification, and currently enrolled patients must re-consent to trial participation.

Non-substantial/material changes, e.g., minor corrections of administrative nature and/or rephrasing, which do not meet the above criteria for being substantial, are considered editorial changes. Such minor corrections will be reported to the IEC and CA (in EU via CTIS) together with the next substantial modification.

If new events occur related to the conduct of the trial or the development of the IP, which may affect the patients' safety, the sponsor and the investigator will take appropriate safety measures to protect the patients from any immediate hazard. The sponsor will immediately inform the CA and IEC of the new events and the measures taken.

21.2 Protocol deviations

A PD is a failure to follow, intentionally or unintentionally, the requirements of the protocol.

In emergency circumstances, deviations from the protocol may proceed without prior consultation with the sponsor and favorable opinion of the IEC, if the rights, safety, and well-being of human trial participants need to be protected. Such deviations will be documented and reported to the sponsor and the IEC as soon as possible in accordance with national regulations.

All PDs will be listed. The evaluability of concerned patients for final data analysis will be discussed in a blinded data review meeting held prior to statistical analyses.

For reporting of a serious deviation please see Section [22.6](#).

21.3 Data retention

The sponsor's designee will provide an investigator site file (ISF) to each center. The ISF will include essential documents as defined by the ICH GCP guideline and applicable local requirements.

The investigator will be responsible to update and maintain the ISF, which will be reviewed periodically by the monitor(s). These documents may be reviewed during an audit by the sponsor or an inspection by the CA.

All essential documents at the trial center, the sponsor or sponsor's designee should be retained until at least 2 years after the last approval of a marketing application in an ICH region, until there are no pending or contemplated marketing applications in an ICH region, and until at least 2 years have elapsed since the formal discontinuation of clinical development of the IP and at least 25 years after the end of trial, whichever period is longer. The final report will be kept for another 5 years after the IP has been taken from the market according to the legal stipulations.

The documents should be archived for a longer period, if required by the applicable regulatory authorities or if agreed with the sponsor. The sponsor is responsible to inform the investigators when these documents have to be retained no longer.

The medical files of trial patients must be retained in accordance with local legislation and in accordance with the maximum period of time permitted by the hospital, institution, or private practice.

22 Data collection, monitoring, and quality assurance

22.1 Data collection

All data generated after the patient has given informed consent, except for PK, ADA, and biomarker data, must be recorded in the eCRF in a timely manner. The investigator will be appropriately trained in the use of the eCRF system (viedocTM), e.g., at the investigator meeting and site initiation visit. The investigator must ensure accurate and proper completion of the eCRF.

No data will be recorded directly in the eCRF without records in source data documents.

Definitions on what constitutes source data can be found in the monitoring plan.

Only investigators and authorized designees will enter and edit data in the eCRF via a secure network and a secure access system. Completed data for each visit will be approved by the investigator or authorized designee using an electronic signature to confirm the accuracy of the data. Any change or addition will be recorded by an electronic audit trail system. A list of all persons who are allowed to make entries in the eCRF must be available at each trial center. The investigator must verify that all data entries in the eCRF are accurate and correct. Entries will be checked against appropriate source documentation by the monitor (Section 22.2).

22.2 Monitoring

The extent of monitoring and source data verification will be specified in a monitoring plan.

The trial center may not obtain consent of any patient before the initiation visit. During the trial further monitoring visits will be performed according to ICH GCP, the sponsor's designee's standard operating procedures (SOPs), and local regulations. eCRFs will be reviewed against source data for adherence to the trial protocol and ICH GCP, as well as for completeness, accuracy, and consistency of data. Additionally, the monitor will check the progress of enrollment, and will ensure that the IP is being stored, dispensed, and accounted for according to specifications. Key trial personnel must be available to assist the monitor during these visits.

Risk-based monitoring including reduced source data verification, triggered monitoring, centralized monitoring, and targeted monitoring may be conducted.[62,63,64,65] The extent and details of these monitoring methods will be defined in the monitoring plan.

The investigator must allow the monitor access to the patient's medical records and all applicable source documents. Throughout the trial, all data captured in the eCRF will only be identified by a patient number. The data will be pseudonymized correspondingly in all data analyses.

The investigator is obligated to assure documentation of all relevant data in the patient file, such as medical history and concomitant diseases, date of written informed consent, visit dates, results of examinations, administrations of medication, and AEs.

Should physical monitoring visits at the center not be feasible or restricted for some time due to an epidemic situation, either a combined remote and on-site monitoring or full remote monitoring visit may be conducted during these periods. In line with local laws and regulations remote source data verification may be part of the remote monitoring visits and if so, has to be agreed between the sponsor's designee and the trial center. If necessary, the relevant study documents (e.g., monitoring plan) will be adjusted to reflect these activities.

Options for remote monitoring - in compliance with the local, country specific regulations, requirements, and relevant guidelines - might be telephone calls, audio/videoconferencing, and sharing of medical records (e.g., direct access to Electronic Health Records [66,67] sharing

redacted/blinded medical records in a secured way, etc.), depending on the center's capability to contribute.

The ICH-GCP requirements and applicable data protection and privacy regulations must be met in any case and for any selected monitoring approach. Remote monitoring of data must be agreed to by the patients in the ICF.

22.3 Audits and inspection

During the trial, audits may be performed by independent auditors, the sponsor, or representatives of the sponsor. Audits of clinical research activities will be performed in accordance with corresponding SOPs to ensure compliance with the principles of GCP.

Regulatory authorities may wish to conduct an inspection. If an inspection is requested, the investigator must inform the sponsor or the sponsor's designee immediately.

The investigator must allow the persons performing the audits or inspections access to source data and documents and will answer any questions.

Inspections by CA are possible at any time, even after the end of the trial.

22.4 Data management procedures

Data management activities will be conducted by the sponsor's designee following their SOPs. The database will be built by the sponsor's designee.

Details on data handling will be described in the data management plan. Data entered into the eCRF will be validated through online edit checks and offline checks run by the data manager or designee according to the data validation plan. All identified discrepancies will be queried and addressed to the investigator.

The sponsor's designee will handle the data cleaning process, query process, and coding.

The database will be soft locked when the last patient has completed the last visit and when it is considered complete and accurate (i.e., all data cleaning activities performed). The database will be hard locked after all the changes following the blind data review meeting have been done and the database is considered complete and accurate. All changes will be tracked (audit trail). The sponsor's approval prior to database hard lock is mandatory.

MedDRA will be used for coding of AEs and medical history. Concomitant medication will be coded using the World Health Organization Drug Dictionary Anatomical Therapeutical Chemical code.

22.5 Training of center staff (optional)

The investigator will ensure that everyone assisting with the clinical trial is adequately informed about the protocol, the IP, and his/her study-related duties and functions. Furthermore, the investigator will maintain a list of qualified persons to whom the investigator has delegated trial-related duties.

22.6 Serious breaches

Concerning the definition of a serious breach, the applicable legislation must be adhered to and guidance relevant to the concerned countries must be consulted.

If a serious breach is suspected, the sponsor and sponsor's designee must be contacted within 1 working day by telephone or email. Details of the relevant person(s) to be contacted can be found in the contact list provided in the ISF. In collaboration with the investigator and sponsor's designee the serious breach will be reviewed by the sponsor and, if appropriate, the sponsor or sponsor's designee will notify the competent authorities and IECs (in the EU member states concerned through CTIS), if applicable, about the serious breach without undue delay but not later than 7 calendar days of becoming aware of that breach.

23 Trial report and publications

The trial will be registered before the inclusion of the first patient in a public register according to local regulations.

After completion of the trial, the results will be summarized in a clinical trial report according to the ICH E3 Note for guidance on structure and content of clinical trial reports. The results of the PK, ADA, and biomarker analyses will not be included in the clinical trial report and reported separately.

Within 1 year from the end of the trial, the sponsor or the sponsor's designee will submit a summary of the results of the clinical trial and, where appropriate, a summary written in a manner that is understandable to laypersons, in accordance with the relevant national provisions and, where applicable, the Clinical Trials Regulation (EU) No 536/2014.

The main findings of the clinical trial will be published.

By signing the trial protocol, the investigator agrees with the use of results of the trial for the purposes of national and international registration, publication, and information for medical and pharmaceutical professionals. If necessary, the authorities will be notified of the investigator's name, address, qualifications, and extent of involvement.

No data resulting from the trial will be presented or published in any form or media by the institution, investigator, or research staff without the prior written consent of the sponsor. Investigators should submit all manuscripts or abstracts to the sponsor for its prior written approval at least 60 days prior to submission. In addition, as the trial is a multi-center clinical trial, no individual publications will be made until the first multi-center publication or presentation. Subsequent publications must reference the primary publication and all publications will acknowledge all trial sites.

Authorship will be determined by mutual agreement between the TSC and potential co-authors based on relevant contribution in accordance with international scientific and ethical standards, including the International Committee of Medical Journal Editors authorship requirements

The preparation and submission of abstracts or manuscripts including the study results must be in line with the process specified in the investigator's clinical trial agreement. The publication or presentation of any study results shall comply with all applicable privacy laws.

24 Trial periods

Trial start (first patient first visit):	Q4 2023
Estimated trial end (last patient last visit):	Q4 2025
Estimated recruitment period:	21 months
Expected duration of patient participation:	about 3 months (including Screening)

The end of the trial is defined as last patient visit in any participating center.

25 References

1. Inker LA, Eneanya ND, Coresh J, Tighiouart H, Wang D, Sang Y, et al. New Creatinine- and Cystatin C-Based Equations to Estimate GFR without Race. *N Engl J Med*. 2021;385(19):1737-49.
2. Chew STH, Hwang NC. Acute Kidney Injury After Cardiac Surgery: A Narrative Review of the Literature. *J Cardiothorac Vasc Anesth*. 2019;33(4):1122-38.
3. Matsuura R, Iwagami M, Moriya H, Ohtake T, Hamasaki Y, Nangaku M, et al. The Clinical Course of Acute Kidney Disease after Cardiac Surgery: A Retrospective Observational Study. *Sci Rep*. 2020;10(1):6490.
4. Nadim MK, Forni LG, Bihorac A, Hobson C, Koyner JL, Shaw A, et al. Cardiac and Vascular Surgery-Associated Acute Kidney Injury: The 20th International Consensus Conference of the ADQI (Acute Disease Quality Initiative) Group. *J Am Heart Assoc*. 2018;7(11).
5. Vives M, Hernandez A, Parramon F, Estanyol N, Pardina B, Muñoz A, et al. Acute kidney injury after cardiac surgery: prevalence, impact and management challenges. *Int J Nephrol Renovasc Dis*. 2019;12:153-66.
6. Wang Y, Bellomo R. Cardiac surgery-associated acute kidney injury: risk factors, pathophysiology and treatment. *Nat Rev Nephrol*. 2017;13(11):697-711.
7. Choe SH, Cho H, Bae J, Ji SH, Yoon HK, Lee HJ, et al. Severity and Duration of Acute Kidney Injury and Chronic Kidney Disease after Cardiac Surgery. *J Clin Med*. 2021;10(8).
8. Kellum JA, Lameire N. Diagnosis, evaluation, and management of acute kidney injury: a KDIGO summary (Part 1). *Crit Care*. 2013;17(1):204.
9. Rosner MH, Okusa MD. Acute kidney injury associated with cardiac surgery. *Clin J Am Soc Nephrol*. 2006;1(1):19-32.
10. Priyanka P, Zarbock A, Izawa J, Gleason TG, Renfurm RW, Kellum JA. The impact of acute kidney injury by serum creatinine or urine output criteria on major adverse kidney events in cardiac surgery patients. *J Thorac Cardiovasc Surg*. 2021;162(1):143-51.e7.
11. Howitt SH, Grant SW, Caiado C, Carlson E, Kwon D, Dimarakis I, et al. The KDIGO acute kidney injury guidelines for cardiac surgery patients in critical care: a validation study. *BMC Nephrol*. 2018;19(1):149.
12. O'Neal JB, Shaw AD, Billings FTt. Acute kidney injury following cardiac surgery: current understanding and future directions. *Crit Care*. 2016;20(1):187.
13. Thakar CV, Arrigain S, Worley S, Yared JP, Paganini EP. A clinical score to predict acute renal failure after cardiac surgery. *J Am Soc Nephrol*. 2005;16(1):162-8.
14. Charytan DM, Zelevinsky K, Wolf R, Normand ST. All-Cause Mortality and Progression to End-Stage Kidney Disease Following Percutaneous Revascularization or Surgical Coronary Revascularization in Patients with CKD. *Kidney Int Rep*. 2021;6(6):1580-91.
15. Karim HM, Yunus M, Saikia MK, Kalita JP, Mandal M. Incidence and progression of cardiac surgery-associated acute kidney injury and its relationship with bypass and cross clamp time. *Ann Card Anaesth*. 2017;20(1):22-7.
16. Prowle JR, Kolic I, Purdell-Lewis J, Taylor R, Pearse RM, Kirwan CJ. Serum creatinine changes associated with critical illness and detection of persistent renal dysfunction after AKI. *Clin J Am Soc Nephrol*. 2014;9(6):1015-23.

17. Casas A, Mallén A, Blasco-Lucas A, Sbraga F, Guiteras J, Bolaños N, et al. Chronic Kidney Disease-Associated Inflammation Increases the Risks of Acute Kidney Injury and Mortality after Cardiac Surgery. *Int J Mol Sci.* 2020;21(24).
18. Abebe A, Kumela K, Belay M, Kebede B, Wobie Y. Mortality and predictors of acute kidney injury in adults: a hospital-based prospective observational study. *Sci Rep.* 2021;11(1):15672.
19. Basile DP, Bonventre JV, Mehta R, Nangaku M, Unwin R, Rosner MH, et al. Progression after AKI: Understanding Maladaptive Repair Processes to Predict and Identify Therapeutic Treatments. *J Am Soc Nephrol.* 2016;27(3):687-97.
20. Meersch M, Schmidt C, Hoffmeier A, Van Aken H, Wempe C, Gerss J, et al. Prevention of cardiac surgery-associated AKI by implementing the KDIGO guidelines in high risk patients identified by biomarkers: the PrevAKI randomized controlled trial. *Intensive Care Med.* 2017;43(11):1551-61.
21. Zarbock A, Küllmar M, Ostermann M, Lucchese G, Baig K, Cennamo A, et al. Prevention of Cardiac Surgery-Associated Acute Kidney Injury by Implementing the KDIGO Guidelines in High-Risk Patients Identified by Biomarkers: The PrevAKI-Multicenter Randomized Controlled Trial. *Anesth Analg.* 2021;133(2):292-302.
22. Peters E, Ergin B, Kandil A, Gurel-Gurevin E, van Elsas A, Masereeuw R, et al. Effects of a human recombinant alkaline phosphatase on renal hemodynamics, oxygenation and inflammation in two models of acute kidney injury. *Toxicol Appl Pharmacol.* 2016;313:88-96.
23. Peters E, Geraci S, Heemskerk S, Wilmer MJ, Bilos A, Kraenzlin B, et al. Alkaline phosphatase protects against renal inflammation through dephosphorylation of lipopolysaccharide and adenosine triphosphate. *Br J Pharmacol.* 2015;172(20):4932-45.
24. Neyra JA, Hu MC, Minhajuddin A, Nelson GE, Ahsan SA, Toto RD, et al. Kidney Tubular Damage and Functional Biomarkers in Acute Kidney Injury Following Cardiac Surgery. *Kidney Int Rep.* 2019;4(8):1131-42.
25. Peters E, van Elsas A, Heemskerk S, Jonk L, van der Hoeven J, Arend J, et al. Alkaline phosphatase as a treatment of sepsis-associated acute kidney injury. *J Pharmacol Exp Ther.* 2013;344(1):2-7.
26. Humphreys BD, Cantaluppi V, Portilla D, Singbartl K, Yang L, Rosner MH, et al. Targeting Endogenous Repair Pathways after AKI. *J Am Soc Nephrol.* 2016;27(4):990-8.
27. Peters E, Arend J, Tiessen R, Van Elsas A, Masereeuw R, Pickkers P. Pharmacokinetics, safety and tolerability of human recombinant alkaline phosphatase in healthy volunteers. *Crit Care.* 2015;19(Suppl 1):P126.
28. Powles T, Bellmunt J, Comperat E, De Santis M, Huddart R, Loriot Y, et al. Bladder cancer: ESMO Clinical Practice Guideline for diagnosis, treatment and follow-up. *Ann Oncol.* 2022;33(3):244-58.
29. Pickkers P, Mehta RL, Murray PT, Joannidis M, Molitoris BA, Kellum JA, et al. Effect of Human Recombinant Alkaline Phosphatase on 7-Day Creatinine Clearance in Patients With Sepsis-Associated Acute Kidney Injury: A Randomized Clinical Trial. *Jama.* 2018;320(19):1998-2009.
30. Siew ED, Abdel-Kader K, Perkins AM, Greevy RA, Jr., Parr SK, Horner J, et al. Timing of Recovery From Moderate to Severe AKI and the Risk for Future Loss of Kidney Function. *Am J Kidney Dis.* 2020;75(2):204-13.

31. Chawla LS, Bellomo R, Bihorac A, Goldstein SL, Siew ED, Bagshaw SM, et al. Acute kidney disease and renal recovery: consensus report of the Acute Disease Quality Initiative (ADQI) 16 Workgroup. *Nat Rev Nephrol*. 2017;13(4):241-57.
32. Rydén L, Sartipy U, Evans M, Holzmänn MJ. Acute kidney injury after coronary artery bypass grafting and long-term risk of end-stage renal disease. *Circulation*. 2014;130(23):2005-11.
33. Han SS, Shin N, Baek SH, Ahn SY, Kim DK, Kim S, et al. Effects of acute kidney injury and chronic kidney disease on long-term mortality after coronary artery bypass grafting. *Am Heart J*. 2015;169(3):419-25.
34. Linder A, Fjell C, Levin A, Walley KR, Russell JA, Boyd JH. Small acute increases in serum creatinine are associated with decreased long-term survival in the critically ill. *Am J Respir Crit Care Med*. 2014;189(9):1075-81.
35. van Groenendaal R, Beunders R, Hemelaar P, Hofland J, Morshuis WJ, van der Hoeven JG, et al. Safety and Efficacy of Human Chorionic Gonadotropin Hormone-Derivative EA-230 in Cardiac Surgery Patients: A Randomized Double-Blind Placebo-Controlled Study. *Crit Care Med*. 2021;49(5):790-803.
36. Thielmann M, Corteveille D, Szabo G, Swaminathan M, Lamy A, Lehner LJ, et al. Teprasiran, a Small Interfering RNA, for the Prevention of Acute Kidney Injury in High-Risk Patients Undergoing Cardiac Surgery: A Randomized Clinical Study. *Circulation*. 2021;144(14):1133-44.
37. McCullough PA, Bennett-Guerrero E, Chawla LS, Beaver T, Mehta RL, Molitoris BA, et al. ABT-719 for the Prevention of Acute Kidney Injury in Patients Undergoing High-Risk Cardiac Surgery: A Randomized Phase 2b Clinical Trial. *J Am Heart Assoc*. 2016;5(8).
38. Ejaz AA, Martin TD, Johnson RJ, Winterstein AG, Klodell CT, Hess PJ, Jr., et al. Prophylactic nesiritide does not prevent dialysis or all-cause mortality in patients undergoing high-risk cardiac surgery. *J Thorac Cardiovasc Surg*. 2009;138(4):959-64.
39. Singh I, Rajagopalan S, Srinivasan A, Achuthan S, Dhamija P, Hota D, et al. Preoperative statin therapy is associated with lower requirement of renal replacement therapy in patients undergoing cardiac surgery: a meta-analysis of observational studies. *Interact Cardiovasc Thorac Surg*. 2013;17(2):345-52.
40. Swaminathan M, Stafford-Smith M, Chertow GM, Warnock DG, Paragamian V, Brenner RM, et al. Allogeneic Mesenchymal Stem Cells for Treatment of AKI after Cardiac Surgery. *J Am Soc Nephrol*. 2018;29(1):260-7.
41. Hillis GS, Croal BL, Buchan KG, El-Shafei H, Gibson G, Jeffrey RR, et al. Renal function and outcome from coronary artery bypass grafting: impact on mortality after a 2.3-year follow-up. *Circulation*. 2006;113(8):1056-62.
42. Gibson PH, Croal BL, Cuthbertson BH, Chiwara M, Scott AE, Buchan KG, et al. The relationship between renal function and outcome from heart valve surgery. *Am Heart J*. 2008;156(5):893-9.
43. Davidson JA, Khailova L, Treece A, Robison J, Soranno DE, Jaggars J, et al. Alkaline Phosphatase Treatment of Acute Kidney Injury in an Infant Piglet Model of Cardiopulmonary Bypass with Deep Hypothermic Circulatory Arrest. *Sci Rep*. 2019;9(1):14175.

44. Davidson JA, Frank BS, Urban TT, Twite M, Jagers J, Khailova L, et al. Serum metabolic profile of postoperative acute kidney injury following infant cardiac surgery with cardiopulmonary bypass. *Pediatr Nephrol*. 2021;36(10):3259-69.
45. Thakar CV. Perioperative acute kidney injury. *Adv Chronic Kidney Dis*. 2013;20(1):67-75.
46. Nashef SA, Roques F, Sharples LD, Nilsson J, Smith C, Goldstone AR, et al. EuroSCORE II. *Eur J Cardiothorac Surg*. 2012;41(4):734-44; discussion 44-5.
47. Ruggieri VG, Bounader K, Verhoye JP, Onorati F, Rubino AS, Gatti G, et al. Prognostic Impact of Prolonged Cross-Clamp Time in Coronary Artery Bypass Grafting. *Heart Lung Circ*. 2018;27(12):1476-82.
48. Head SJ, Milojevic M, Taggart DP, Puskas JD. Current Practice of State-of-the-Art Surgical Coronary Revascularization. *Circulation*. 2017;136(14):1331-45.
49. Prischl FC, Rossing P, Bakris G, Mayer G, Wanner C. Major adverse renal events (MARE): a proposal to unify renal endpoints. *Nephrol Dial Transplant*. 2021;36(3):491-7.
50. Forni LG, Darmon M, Ostermann M, Oudemans-van Straaten HM, Pettilä V, Prowle JR, et al. Renal recovery after acute kidney injury. *Intensive Care Med*. 2017;43(6):855-66.
51. Oshima M, Toyama T, Haneda M, Furuichi K, Babazono T, Yokoyama H, et al. Estimated glomerular filtration rate decline and risk of end-stage renal disease in type 2 diabetes. *PLoS One*. 2018;13(8):e0201535.
52. James MT, Grams ME, Woodward M, Elley CR, Green JA, Wheeler DC, et al. A Meta-analysis of the Association of Estimated GFR, Albuminuria, Diabetes Mellitus, and Hypertension With Acute Kidney Injury. *Am J Kidney Dis*. 2015;66(4):602-12.
53. Peters E, Heuberger J, Tiessen R, van Elsas A, Masereeuw R, Arend J, et al. Pharmacokinetic Modeling and Dose Selection in a Randomized, Double-Blind, Placebo-Controlled Trial of a Human Recombinant Alkaline Phosphatase in Healthy Volunteers. *Clin Pharmacokinet*. 2016;55(10):1227-37.
54. Peters E, Mehta RL, Murray PT, Hummel J, Joannidis M, Kellum JA, et al. Study protocol for a multicentre randomised controlled trial: Safety, Tolerability, efficacy and quality of life Of a human recombinant alkaline Phosphatase in patients with sepsis-associated Acute Kidney Injury (STOP-AKI). *BMJ Open*. 2016;6(9):e012371.
55. Moh'd AF, Al-Odwan HT, Altarabsheh S, Makahleh ZM, Khasawneh MA. Predictors of aortic clamp time duration and intensive care unit length of stay in elective adult cardiac surgery. *Egypt Heart J*. 2021;73(1):92.
56. Nadeem R, Agarwal S, Jawed S, Yasser A, Altahmody K. Impact of Cardiopulmonary Bypass Time on Postoperative Duration of Mechanical Ventilation in Patients Undergoing Cardiovascular Surgeries: A Systemic Review and Regression of Metadata. *Cureus*. 2019;11(11):e6088.
57. Khwaja A. KDIGO clinical practice guidelines for acute kidney injury. *Nephron Clin Pract*. 2012;120(4):c179-84.
58. Schneider AG, Eastwood GM, Seevanayagam S, Matalanis G, Bellomo R. A risk, injury, failure, loss, and end-stage renal failure score-based trigger for renal replacement therapy and survival after cardiac surgery. *J Crit Care*. 2012;27(5):488-95.
59. Roques F, Michel P, Goldstone AR, Nashef SA. The logistic EuroSCORE. *Eur Heart J*. 2003;24(9):881-2.

60. EU. Regulation (EU) No 536/2014 of the European parliament and of the council of 16 April 2014 on clinical trials on medicinal products for human use, and repealing Directive 2001/20/EC. Published 2014. Accessed in Oct 2022. Available at: <https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex%3A32014R0536>.
61. EU. Regulation (EU) 2016/679 of the European Parliament and of the Council of 27 April 2016 on the protection of natural persons with regard to the processing of personal data and on the free movement of such data, and repealing Directive 95/46/EC (General Data Protection Regulation). Published 2016. Accessed in Oct 2022. Available at: <https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:32016R0679>.
62. EMA. Reflection paper on risk based quality management in clinical trials (EMA/269011/2013). Published 2013. Accessed in Oct 2022. Available at: https://www.ema.europa.eu/en/documents/scientific-guideline/reflection-paper-risk-based-quality-management-clinical-trials_en.pdf.
63. FDA. Guidance on Conduct of Clinical Trials of Medical Products during COVID-19 Pandemic, Guidance for Industry, Investigators, and Institutional Review Boards. Published 2021. Accessed in Oct 2022. Available at: <https://www.fda.gov/regulatory-information/search-fda-guidance-documents/fda-guidance-conduct-clinical-trials-medical-products-during-covid-19-public-health-emergency>.
64. FDA. Guidance for Industry - Oversight of Clinical Investigations - A Risk-Based Approach to Monitoring. Published 2013. Accessed in Oct 2022. Available at: <https://www.fda.gov/media/116754/download>.
65. EMA. Guidance on the Management of Clinical Trials during the COVID-19 (Coronavirus) pandemic. Volume 5. Published 2022. Accessed in Oct 2022. Available at: https://ec.europa.eu/health/sites/health/files/files/eudralex/vol-10/guidanceclinicaltrials_covid19_en.pdf.
66. EMA. Guideline on computerised systems and electronic data in clinical trials (EMA/INS/GCP/112288/2023). 9 March 2023. Published 2023. Accessed in Mar 2023. Available at: https://www.ema.europa.eu/en/documents/regulatory-procedural-guideline/guideline-computerised-systems-electronic-data-clinical-trials_en.pdf.
67. FDA. Guidance for Industry, Use of Electronic Health Record Data in Clinical Investigations Published 2018. Accessed in Oct 2022. Available at: <https://www.fda.gov/regulatory-information/search-fda-guidance-documents/use-electronic-health-record-data-clinical-investigations-guidance-industry>.

26 Approval and signatures

Protocol agreed to by sponsor:



Sponsor's signatory (name in print)

Signature

Date



Sponsor's signatory (name in print)

Signature

Date

Protocol agreed to by coordinating investigator:

Peter Pickkers

Coordinating investigator (name in print)

Signature

Date

Confidential

Principal investigator agreement page for the protocol

I agree:

To assume responsibility for the proper conduct of the clinical study at this site, and to conduct the trial in compliance with national law, the current version of the Declaration of Helsinki, the Clinical Trials Regulation (EU) No 536/2014, the ICH guideline for GCP (CPMP/ICH/135/95), the present trial protocol including its modifications, and with any other trial conduct procedures provided by the sponsor or authorized representatives.

Not to implement any deviations from or changes to the protocol (including protocol modifications) without agreement from the sponsor and prior review and favorable opinion from the IEC and approval from the CA, if applicable, except where necessary to eliminate an immediate hazard to the patient(s), or for administrative aspects of the clinical trial (where permitted by all applicable regulatory requirements).

That I am familiar with the appropriate use of the IP as described in this protocol and any other information provided by the sponsor including, but not limited to, the current IB or equivalent document provided by the sponsor.

To ensure that all persons assisting me with the clinical trial are adequately informed about the IP and of their trial-related duties and functions.

That I have been informed that certain regulatory authorities and Clinical Trials Regulation (EU) No 536/2014 require the sponsor to obtain and supply details about the investigator's interest, such as economic interests, institutional affiliations, or personal interests, which may influence the investigator's impartiality. The sponsor will use and disclose the information solely for the purpose of complying with regulatory requirements.

Principal investigator (name in print)

Signature

Date

Confidential

27 Appendices

Appendix 1 Common surgical complications

Procedure, the complications listed are considered common surgical complications and on occurrence and meeting the described definition can be documented on the checklist in the electronic case record form if the principle investigator deems this appropriate.

1. Mild Acute Respiratory Distress Syndrome (ARDS) a. Definition. Following the Berlin definition of respiratory distress syndrome¹, with PaO₂:FIO₂ between 26.7 and 40.0 kPa (200–300mmHg) with PEEP or CPAP 5 cmH₂O.

2. Mild to moderate Arrhythmia a. Definition. electrocardiograph (ECG) evidence of cardiac rhythm disturbance.

b. Severity grading (1) Mild. Results in only temporary harm and would not usually require specific clinical treatment.

(2) Moderate. More serious complication but one which does not usually result in permanent harm or functional limitation. Usually requires clinical treatment.

3. Mild Cardiogenic pulmonary oedema a. Definition. evidence of fluid accumulation in the alveoli due to poor cardiac function that results in only temporary harm and would not usually require specific clinical treatment.

4. Mild Deep vein thrombosis (DVT) a. Definition. A new blood clot or thrombus within the venous system that results in only temporary harm and would not usually require specific clinical treatment.

5. Mild Delirium a. Definition. Delirium may be identified using the Intensive Care Delirium Screening Checklist². Patients are first evaluated for an altered level of consciousness. Those with a response to mild or moderate stimulation, an exaggerated response to stimulation or normal wakefulness are evaluated fully. Patients receive one point for each of the following criteria: inattention, disorientation, hallucination-delusion-psychosis, psychomotor agitation or retardation, inappropriate speech or mood, sleep/wake cycle disturbance or symptom fluctuation. Mild delirium is diagnosed with a score of 2 or 3.

6. Mild Gastrointestinal bleed a. Definition. unambiguous clinical or endoscopic evidence of blood in the gastrointestinal tract that results in only temporary harm and would not usually require specific clinical treatment.

7. Mild Myocardial infarction a. Definition. Increase in serum cardiac biomarker values (preferably cardiac troponin) with at least one value above the 99th percentile upper reference limit and at least one of the following criteria: symptoms of ischemia; new or presumed new significant ST segment or T wave ECG changes or new left bundle branch block; development of pathological Q waves on ECG; radiological or echocardiographic evidence of

new loss of viable myocardium or new regional wall motion abnormality; identification of an intracoronary thrombus at angiography or autopsy. Event results in only temporary harm and would not usually require specific clinical treatment.

8. Mild Paralytic ileus a. Definition. Failure to tolerate solid food or defecate for three or more days after surgery that results in only temporary harm and would not usually require specific clinical treatment.

9. Mild Surgical site infection

a. Definition.

- (1) Infection occurs within 30 days after surgery and
- (2) Involves only skin and subcutaneous tissue of the incision and
- (3) The patient has at least one of the following:
 - (a) purulent drainage from the superficial incision
 - (b) organisms isolated from an aseptically obtained culture of fluid or tissue from the superficial incision
 - (c) at least one of the following symptoms or signs of infection: pain or tenderness, localised swelling, redness or heat, and superficial incision is deliberately opened by surgeon and is culture positive or not cultured. A culture negative finding does not meet this criterion.
 - (d) diagnosis of an incisional surgical site infection by a surgeon or attending physician.

b. Severity grading. Results in only temporary harm and would not usually require specific clinical treatment.

10. Mild Urinary tract infection a. Definition. a positive urine culture of 10⁵ colony forming units/ml with no more than two species of micro-organisms, and with at least one of the following symptoms or signs: fever (>38.8°C), urgency, frequency, dysuria, suprapubic tenderness, costovertebral angle pain or tenderness with no other recognized cause that results in only temporary harm and would not usually require specific clinical treatment.

11. Mild Hypovolemia, a. Definition. Need for fluid suppletion more than 3 liters but no more than 6 liters in first 24 hrs.

12. Need for prolonged hemodynamic support. a. Definition. norepinephrine (or equivalent) infusion rate, e.g. above 0.3 ug/kg/min or for more than 24 but less than 72 hours post-surgery.

13. Need for prolonged mechanical ventilation. a. Definition. Need for mechanical ventilation for more than 12 hours but less than 24 hours after ICU admission.

14. Mild blood loss. a. Definition. Blood loss in during and in the first 48 hours of surgery, more than 500cc but no more than 3000 cc.

References

1. Force, A., Ranieri, V. & Rubenfeld, G. Acute Respiratory Distress Syndrome: The Berlin Definition. *JAMA* **307**, (2012).
2. Bergeron, N., Dubois, M.-J., Dumont, M., Dial, S. & Skrobik, Y. Intensive Care Delirium Screening Checklist: evaluation of a new screening tool. *Intensive Care Med.* **27**, 859–864 (2001).