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Abstract

Introduction

Acute kidney injury (AKI) is a common complication after cardiac surgery and is associated with adverse short- and long-term outcomes. Alpha-1-microglobulin (A1M) is a circulating glycoprotein with antioxidant-, heme-binding and mitochondrial-protective mechanisms. RMC-035 is a modified, more soluble, variant of A1M and has been proposed as a novel targeted therapeutic protein to prevent cardiac surgery-associated AKI (CS-AKI). RMC-035 was considered safe and generally well tolerated when evaluated in four clinical Phase 1 studies.

Methods and Analysis

This is a Phase 2, randomized, double-blind, adaptive design, parallel group clinical study that evaluates RMC-035 compared to placebo in approximately 268 cardiac-surgical patients at high risk for CS-AKI. RMC-035 is administered as an IV infusion. In total, five doses will be given. Dosing is based on pre-surgery eGFR, and will be either 1.3 or 0.65 mg/kg.

The primary study objective is to evaluate whether RMC-035 reduces the incidence of post-operative AKI, and key secondary objectives are to evaluate whether RMC-035 improves post-operative renal function compared to placebo. A blinded interim analysis with potential sample size re-assessment is planned once 134 randomized subjects have completed dosing. An independent Data Monitoring Committee will evaluate safety and efficacy data at pre-specified intervals throughout the trial. The study is a global multi-center study at approximately 30 sites.

Ethics and dissemination

The trial was approved by independent ethics committees/relevant institutional review boards and is conducted in accordance with Good Clinical Practice, the Declaration of Helsinki, and other applicable regulations. Results of this study will be published in a peer-reviewed scientific journal.

Trial registration number

EudraCT Number: 2021-004040-19

ClinicalTrials.gov Identifier: NCT05126303

Keywords

Acute kidney injury, acute renal failure, adult intensive & critical care, A1M, alpha-1-microglobulin, cardiac surgery, RMC-035, randomized controlled trial, cardiac surgery, cardiac surgery-associated AKI, CS-AKI.

Article Summary

Strengths and limitations of this study

- This is a well-controlled and adequately powered Phase 2 trial in cardiac surgery patients at increased risk for acute kidney injury to evaluate the efficacy of RMC-035 on the occurrence of AKI within 3 days after surgery. Results of this trial may also allow for evaluation of other clinically relevant outcomes such as post-operative renal function up to 90 days after surgery.
- The trial was designed by a group of global experts in AKI and cardiac surgery, with input from or review by the US Food and Drug Administration and several national medicinal regulatory authorities in Europe and North America.
- This trial uses an adaptive trial design whilst preserving data integrity based on a blinded interim analysis. This allows for re-assessment of statistical assumptions, e.g. AKI event rate, with the possibility to increase the number of patients to be recruited.
- Trial subjects will be recruited at multiple centers in Europe and North America that are considered to reflect standard-of-care in cardiac surgery.
- Limitation: The trial is not powered to show differences in less frequently occurring longer term outcomes such as major adverse kidney events (MAKE).

Introduction

Globally, an estimated 2 million cardiovascular procedures per year are performed.[1] Acute kidney injury (AKI) is considered an important determinant of mortality in patients undergoing cardiovascular surgery.[2] [3] Various epidemiological studies show that cardiac surgery-associated AKI (CS-AKI) occurs in up to 30% of cardiac surgery patients [4] and is associated with an increased morbidity as well as short- and long-term mortality.[5-8] The short-term and long-term mortality rate are 10.7% and 30% respectively and increase along with severity of stages.[6] Heart failure, chronic hyperglycemia, anemia, obesity, preoperative exposure to nephrotoxic drugs or contrast media, inflammation, proteinuria, and pre-existing kidney disease were systematically reviewed and were found to be associated with an increased risk of postoperative CS-AKI.[9]

In clinical studies evaluating potential treatments to prevent CS-AKI in high-risk groups, event rates of AKI up to 65.5% in a high-risk population [10] and 71.7% in patients with a positive biomarker test (that identifies patients at very high risk for AKI) shortly after surgery [11] have been reported. Recent studies on CS-AKI showed that patient enrichment based on the combination of the complexity of surgery and co-morbidities resulted in AKI rates between 28 and 34.2%.[12 13]

Current treatment strategies and clinical management of CS-AKI are mainly supportive, including optimal fluid management and maintenance of hemodynamic stability.[14] At present, no specific therapeutic interventions are available for prevention or treatment of CS-AKI. This is an area of large unmet clinical need, where patients who develop CS-AKI are at risk for additional severe complications, including need for renal replacement therapy (RRT), development of chronic kidney disease (CKD) and mortality. Thus, a single AKI episode after cardiac surgery can lead to a significant burden of comorbidities, prolonged hospital stays, poor quality of life and high long-term costs.

Recombinant alpha-1-microglobulin (RMC-035)

The investigational medicinal product (IMP), RMC-035, contains the pharmacologically active protein RMC-035, which is a recombinant and modified variant of endogenous human alpha-1-microglobulin (A1M). It has four distinct molecular mechanisms, including heme binding, reductase activity, radical scavenging, and mitochondrial binding/protection.[15] Employing several antioxidative mechanisms, it protects cells and tissues from various forms of cell damage caused by oxidative stress, including free radicals and reactive oxygen species (ROS). [16-19]

RMC-035 has been evaluated in four Phase 1 clinical studies: single ascending dose and multiple ascending dose studies in healthy subjects, a renal impairment study, and a phase 1b

pharmacokinetic and safety study in cardiac surgery patients. Available clinical data indicate that RMC-035 is safe and generally well tolerated across study populations and support its continued development in CS-AKI.

The pharmacological characteristics of RMC-035 have particular translatability to prevention of CS-AKI: it harbors multiple targeted mechanisms against key pathophysiological pathways of CS-AKI, including ischemia-reperfusion injury (IRI), heme toxicity and mitochondrial dysfunction. Furthermore, RMC-035 is rapidly distributed to kidney proximal tubular cells (via glomerular filtration), resulting in high drug exposure in the cells where the primary injury occurs in AKI. It is administered as intravenous (IV) infusion with short systemic half-life which is ideal for hospitalized patients requiring specific renal protection.

Trial objectives

This is a Phase 2, randomized, placebo-controlled, double-blind, adaptive design, parallel group clinical study to evaluate the efficacy and safety of RMC-035 in subjects at high risk for AKI following open-chest cardiac surgery. The study is being conducted to provide proof-of-concept efficacy data and to guide the design of a subsequent pivotal study in this patient population. Based on an integrated assessment of available non-clinical and clinical data, RMC-035 is considered to be well tolerated and is expected to provide targeted renal protection in this patient population, with the aim to prevent or ameliorate peri-operative and post-surgery renal injury.

Primary Objectives:

- To evaluate the efficacy of RMC-035 for prevention of AKI (Kidney Disease Improving Global Outcomes [KDIGO] definition [20] in subjects undergoing coronary artery by-pass graft (CABG) and/or valve surgery and/or aorta surgery with additional risk factors for developing CS-AKI
- To evaluate the safety and tolerability of RMC-035

Key Secondary Objectives:

- To evaluate RMC-035 for the prevention of post-operative decline (within 72 hours) in renal function
- To evaluate RMC-035 for the reduction of post-operative AKI duration

Other secondary Objectives:

- To evaluate RMC-035 for preserving post-surgery renal function up to Day 90
- To evaluate RMC-035 for the prevention of post-operative dialysis up to Day 90
- To evaluate RMC-035 for the prevention of major adverse kidney events (MAKE) at Days 30

and 90, respectively

- To further evaluate RMC-035 for the
    - o Prevention of AKI within 72 hours (based on cystatin C and/or Urine Output [UO])
    - o Persistence and severity of AKI within 72 hours (based on serum creatinine [SCr] and/or UO or cystatin C and/or UO)
    - o Prevention, persistence, and severity of AKI within 7 days (based on SCr and/or UO or cystatin C and/or UO)
- To evaluate RMC-035 for reducing post-operative albuminuria and proteinuria up to Day 90
- To evaluate the pharmacokinetics of RMC-035
- Identification and characterization of anti-drug-antibodies (ADA) developed after intravenous administration of RMC-035

Exploratory Objectives:

- To evaluate post-baseline changes in kidney and cardiac biomarkers
- To evaluate the length of post-operative stay in Intensive Care Unit (ICU) and overall hospitalization time
- To evaluate health-related Quality of Life

Methods and analysis

Patients

The target patient population is adults undergoing open-chest cardiac surgery, ie, CABG and/or valve surgery and/or aorta surgery with additional predisposing risk factors for CS-AKI. Subjects planned to undergo cardiac surgery will be systematically screened for eligibility. Informed consent will be obtained in all eligible patients before randomization and surgery. Inclusion criteria and exclusion are described in Table 1.

Main outcomes

Primary Endpoints

Primary Efficacy Endpoint

AKI within 72 hours after first dose of IMP based on SCr and/or UO (AKI of any stage/severity according to KDIGO definition, ie, SCr ≥1.5 times baseline, or increase of SCr of ≥0.3 mg/dL [≥26.5 µmol/L], or UO <0.5 mL/kg/h for ≥6 hours)

Primary Safety Endpoint

Nature, frequency and severity of treatment-emergent adverse events (TEAEs; defined as any AE which occur within 72 hours after last IMP administration).

Secondary Endpoints

Key Secondary Endpoints

Time-corrected area under the curve (AUC) of SCr for Day 1 to Day 4 (72 hours after first dose of IMP)

Duration of AKI defined as the number of days meeting the definition of AKI (KDIGO definition) starting within 72 hours after first dose of IMP until resolution

For other Secondary Endpoints and Exploratory Endpoints, please see Table 2.

Trial oversight

A scientific advisory board has been established by the sponsor to facilitate design of the trial, provide medical scientific leadership and make trial-specific recommendations to the sponsor. Likewise, an independent unblinded data monitoring committee (DMC) including clinical expertise in AKI will perform review of safety findings at regular intervals during the study. The DMC evaluation will be based on all available accumulated safety data. The DMC will also be responsible for recommendation on the study conduct following the interim analysis (IA). Communication from the DMC will be blinded. All DMC activities and processes will be outlined in a separate DMC Charter.

Patient and public involvement statement

Reflecting the acute and unpredictable nature of CS-AKI there are no specific patient advocacy groups at present; closest are organizations that represent kidney diseases after cardiac surgery in general and, although they are aware of our program, they were not specifically consulted on the design of this study.

Trial design

Overall study design

This is a Phase 2, multi-center, randomized, double-blind, placebo-controlled, adaptive design, parallel group trial including patients undergoing open-chest cardiac surgery. The study consists of a screening period of up to 30 days before surgery, five in-hospital visits during the anticipated post-operative hospitalization period, and two additional follow-up visits at Day 30 and Day 90, respectively. A study flow chart is shown in Figure 1 and the schedule of study specific assessments is presented in Table 3.

Randomization procedure

The trial population consists of patients at high risk for AKI and undergo cardiac surgery with additional predisposing risk factors for CS-AKI (inclusion and exclusion criteria are shown in

Table 1). Eligible subjects will be randomized to receive either RMC-035 or placebo in a 1:1 randomization ratio. Both region (EU versus North America) and pre-operative eGFR calculated using the CKD-EPI equation with SCr on local laboratory results (≥60 and <60 mL/min/1.73m^2) will be used as stratification factors to ensure a balanced randomization within these groups.

IMP dosing

In total five IV doses of either RMC-035 or placebo will be administered during the study. The first dose will start approximately 10 minutes before cardiopulmonary surgery is initiated (0h), and subsequent doses will be given at 6, 12, 24, and 48 hours later. IMP will be given as a continuous IV infusion over either 60 minutes (first two doses) or 30 minutes for subsequent doses. Due to the pharmacokinetic properties of RMC-035, the predefined dose levels (prior to surgery) are based on renal function (eGFR by CKD-EPI equation with SCr) at screening (Day -1, Visit 1): 1) subjects with eGFR ≥60 mL/min/1.73m^2 will receive 1.3 mg/kg (per dose) for the first and second dose, followed by 0.65 mg/kg (per dose) for the third, fourth and fifth dose; 2) Subjects with eGFR >30 and <60 mL/min/1.73m^2 will receive 0.65 mg/kg (per dose) for all five doses. The trial drug is provided on top of standard of care of each center.

Permanent discontinuation of IMP

Local laboratory results will be utilized for clinical care and real time evaluation of AKI for purposes of IMP discontinuation. Subjects who meet any of the following IMP discontinuation criteria during the treatment period will be discontinued from IMP and continue study participation and procedures and follow up as per the schedule of assessment until the end of the study visit: 1) Development of stage ≥2 AKI, according to KDIGO definition, 2) Need for RRT, 3) Need for percutaneous or surgical mechanical circulatory support (MCS) or extra-corporeal membrane oxygenator (ECMO), 4) Reporting of a grade 3 (per Common Terminology Criteria for Adverse Events [CTCAE]) or higher adverse event of ISR or IRR, and which is considered to be an immune-mediated reaction

The IMP will be discontinued in case of any of the following abnormal liver chemistry tests in blood: 1) ALT > 3 × ULN combined with total bilirubin > 2 × ULN in the same sample, 2) ALT > 3 × ULN if associated with symptoms (new or worsening) believed to be related to hepatitis (such as fatigue, nausea, vomiting, right upper quadrant pain or tenderness or jaundice) or believed to be related to hypersensitivity (such as fever, rash or eosinophilia), 3) ALT > 3 x ULN if confirmed in a second sample within 24 hours AND in the second sample International Normalized Ratio (INR) is increased to >1.5 x ULN, 4) ALT > 8 x ULN in any individual sample during the treatment period All liver chemistry abnormalities as summarized above need to be followed up regularly (at least every 24 hours) until values have returned to baseline or are

considered stable. If IMP is discontinued, the subject will remain in the study to be evaluated until the end of the study Visit (as much as possible) or complete the end of the study visit assessments.

Planned sample size & interim analysis (IA)

A minimum of 268 and a maximum of 348 patients are planned to be randomized at approximately 30 sites predominantly across Europe and North America. The final number of patients randomized will depend on the results of the IA.

There will be one IA during the trial which will be conducted once 50% of the planned randomized subjects have completed visit 6 (Day 5-9). Sample size may be increased to a maximum of 348 randomized subject but will not be decreased. The study may be stopped at the IA for futility or safety reasons. Study eligibility criteria may also be modified.

Safety reporting

Safety is followed up until last study visit at Day 90. Serious adverse events (SAEs) will be reported to the sponsor and other stakeholders as per regulatory requirements (eg, competent authorities and/or IRBs/CECs, when applicable). Non-serious AEs will only be reported up to Day 30 Treatment emergent AEs (TEAEs) are defined as AEs which occur after the initial IMP administration through 72 hours after last IMP administration.

Statistical Considerations

For the primary endpoint, KDIGO AKI (serum creatinine and urine output) within 72 hours after first dose of IMP, RMC-035 has been assumed to lead to a 30% relative risk reduction vs. placebo. The event rate in the placebo group has been assumed to be 50%.[21] A sample size of 268 subjects randomized leads to a test power of 80% to show statistically significant results at a two-sided significance level (alpha) of 0.10.

The primary endpoint will be analyzed by the Cochran-Mantel-Haenszel estimate of the common relative risk (RMC-035 vs. placebo) across the four stratification groups formed by region and pre-operative eGFR (≥60 and <60 mL/min/1.73m2). In addition, the proportion of subjects with AKI within 72 hours after first dose of IMP and its 90% confidence interval will be calculated for each treatment group. The hypotheses to be tested are as follows: 1) Null hypothesis: the proportion of subjects developing AKI within 72 hours after first dose of IMP is the same for the RMC-035 and the placebo treatment groups ($\hat{\mathbf{p}}_{\text{RMC-035}} = \hat{\mathbf{p}}_{\text{placebo}}$), 2) Alternative hypothesis: the proportion of subjects developing AKI within 72 hours after first dose of IMP is different for the RMC-035 and the placebo treatment groups ($\hat{\mathbf{p}}_{\text{RMC-035}} \neq \hat{\mathbf{p}}_{\text{placebo}}$).

Secondary continuous efficacy endpoints that are measures of renal function will be analyzed using robust regression of log-transformed values having sequentially imputed any missing

data using multiple imputation. A sensitivity analysis will be performed using a mixed model for repeated measurements (MMRM) if data are approximately normally distributed.

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For the key secondary AUC endpoint, the geometric least square means of the time-corrected AUC of SCr for Days 1 to 4 will be obtained for each, RMC-035 and placebo, by transforming the model estimates back to the original scale.

Supportive analyses of renal function will be performed on cystatin C levels (and corresponding eGFR values assessed by Chronic Kidney Disease Epidemiology Collaboration [CKD-EPI] equations with either SCr, cystatin C, or both) and cumulative AUC values up to each timepoint.

Secondary binary efficacy endpoints will be analyzed using the same approach as specified above for the primary endpoint.

Further details on the IA and the control of the type I error will be provided in the DMC Charter and the Statistical Analysis Plan (SAP).

Safety Endpoints

Safety analyses will be conducted on the Safety Analysis Set (SAF), which includes all randomized subjects who received at least 1 dose of IMP. To characterize the safety profile, the number and percentage of subjects with TEAEs and AEs per dose group will be tabulated. Descriptive statistics will be provided for laboratory tests (hematology, biochemistry, urinalysis) and vital signs (pulse rate, respiratory rate and blood pressure) by visit and for the changes from baseline to each visit split by treatment group. In addition, the development of anti-drug antibodies after intravenous administration of RMC-035 will be investigated and characterized.

Pharmacokinetics

Descriptive statistics will be presented for plasma concentrations by scheduled sample time. Specific pharmacokinetic parameters will be presented separately. Summaries will be provided by dose group, age group and by renal function prior to surgery.

Sensitivity analyses and secondary endpoints

Secondary continuous efficacy endpoints that are measures of renal function will be analyzed using robust regression of log-transformed values having sequentially imputed any missing data using multiple imputation. A sensitivity analysis will be performed using a mixed model for repeated measurements (MMRM) if data are approximately normally distributed.

For the key secondary AUC endpoint, the geometric least square means of the time-corrected

AUC of SCr for Days 1 to 4 will be obtained for each, RMC-035 and placebo, by transforming the model estimates back to the original scale. The relative difference (RMC-035 vs. placebo) and its 90% confidence interval will also be reported.

Supportive analyses of renal function will be performed by using cystatin C levels (and corresponding eGFR values assessed by Chronic Kidney Disease Epidemiology Collaboration [CKD-EPI] equations with either SCr, cystatin C, or both) and cumulative AUC values up to each timepoint.

Secondary binary efficacy endpoints will be analyzed using the same approach as specified above for the primary endpoint.

Additional sensitivity analyses of the primary and secondary endpoints, and evaluation of exploratory endpoints, will be detailed in the SAP.

Ethics and dissemination

This trial will be conducted in accordance with the protocol and consensus ethical principles derived from international guidelines including the Declaration of Helsinki and Council for International Organizations of Medical Sciences (CIOMS) International Ethical Guidelines; applicable International Conference on Harmonization (ICH) GCP Guidelines; and applicable laws and regulations. The protocol, substantial protocol amendments, ICF, IB, and other relevant documents (eg., any other written information regarding this trial to be provided to the patient or the patient's legal representative) will be submitted to an IRB/IEC by the Investigator and reviewed and approved by the IRB/IEC prior to being used in the trial.

Informed consent will be given by patients before any study relevant procedure is performed. Patients will be assigned a unique patient identification number. Any patient records or datasets that are transferred to the Sponsor will contain this identifier only; patient names and any information which would make the patient identifiable will not be transferred. All laboratory specimens, evaluation forms, reports, and other records will be identified in a manner designed to maintain patient confidentiality.

Irrespective of whether the trial is completed or prematurely terminated, the sponsor will ensure that the trial results will be posted on publicly available clinical trial registries in accordance with their requirements. In addition, results will be presented at international congresses and published in peer-reviewed journals.

Conclusions

The ongoing Phase 2 (AKITA) study is specifically designed to answer the primary research question whether the investigational drug RMC-035 can reduce the occurrence of CS-AKI within 3 days after open cardiac surgery. Study results will also provide pertinent information

on other clinically relevant endpoints, such as the severity, duration and persistence of AKI, changes in post-operative renal function and major adverse kidney events (MAKE), and will significantly expand the current knowledge of the safety profile of RMC-035. Finally, biomarker analysis may improve the understanding of its mechanism of action, shed light on relevant disease pathways in CS-AKI and facilitate the design of future clinical trials of RMC-035.

Author's contributions

Alexander Zarbock, Tobias E. Larsson, and Ronny Renfurm co-drafted the MS. All other co-authors participated in trial protocol design, reviewed and edited the manuscript.

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Competing interest statement

Alexander Zarbock received advisory board consultancy and travel reimbursements from Guard Therapeutics.

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Table 1: Inclusion and Exclusion criteria

Inclusion criteria	A subject is eligible for the study if all of the following apply: **1.** Institutional Review Board (IRB)-/Independent Ethics Committee (IEC)-approved written Informed Consent and privacy language as per national regulations has been obtained from the subject prior to any study-related procedures **2.** Subject has the ability to understand and comply with the study requirements and is able to provide written informed consent **3.** Subject age is ≥18 and <85 years **4.** Estimated glomerular filtration rate (eGFR) is ≥30 mL/min/1.73 m2 (at screening) using the Chronic Kidney Disease-Epidemiology Collaboration (CKD-EPI) equation with SCr **5.** Subject is scheduled for non-emergent CABG surgery AND/OR valve surgery (single or multiple valves) AND/OR ascending aorta aneurysm surgery with use of CPB AND AKI risk factors are present (at screening) as specified below: **a.** If only one type of surgery is scheduled at least two AKI risk factors should be present OR eGFR should be <60 mL/min/1.73m2 (at screening) with or without additional risk factors **b.** If any combined surgery is scheduled at least one AKI risk factor should be present Risk factors for AKI are defined below: • Left ventricular ejection fraction (LVEF) <35% at any time during the 3-month period before or at the time of screening as assessed by either echocardiography, cardiac magnetic resonance imaging (MRI) or nuclear scan. • Repeat surgery/history of previous open chest cavity cardiac surgery with or without CPB • Confirmed diagnosis of type 2 diabetes (T2DM) at least 3 months prior to screening AND ongoing treatment with an approved anti-diabetic drug • Age ≥70 years at the time of screening • Heart failure New York Heart Association (NYHA) class II or higher at any time during the 3-month period before or at the time of screening

	• Documented history of previous AKI as per KDIGO criteria longer than 3 months before date of screening independent of the etiology of AKI • Anemia with hemoglobin ≤11 g/dL at any time during the 3-month period before or at the time of screening • Albuminuria, defined as urine albumin-to-creatinine ratio (UACR) >100 mg/g in a spot urine sample or > 100 mg/24 hour in a 24-hour urine collection at any time during the 3-month period before or at the time of screening. • Estimated glomerular filtration rate is <60 mL/min/1.73 m2 using the CKD-EPI equation with SCr at the time of screening **6.** Female subject is either: ***a**. Of non-childbearing potential* • Postmenopausal (defined as at least 1 year without any menses) prior to screening OR • Documented surgically sterile or status post hysterectomy (at least 1 month prior to screening) ***b.** Of childbearing potential* • Agree not to try to become pregnant throughout the study period, or in case of discontinuation, within 28 days after the final Investigational Medicinal Product (IMP) administration • Must have a negative serum pregnancy test at screening • If sexually active, agree to consistently use a highly effective form of birth control starting at screening, and continue to do so throughout the study period, or in case of discontinuation, for 28 days after final IMP administration. If required by local law, 2 highly effective methods of birth control must be used, 1 of which must be a barrier method. **7.** Female subject must not be breastfeeding at screening and throughout the study period, or in case of discontinuation, for 28 days after the final IMP administration **8.** Female subject must not donate ova starting at screening and throughout the study period, or in case of discontinuation, for 28 days after the final IMP administration **9.** Male subject and their female spouse/partner(s) who are of childbearing potential must be using a highly effective form of birth control starting at screening and continue to do so throughout the study period, or in case of discontinuation, up to 12 weeks after final IMP

	administration. If required by local law, 2 highly effective methods of birth control must be used, 1 of which must be a barrier method. **10.** Male subjects must not donate sperm starting from screening, throughout the study period, or in case of discontinuation, up to 12 weeks after final IMP administration **11.** Subject agrees not to participate in another interventional study from the time of signing the informed consent until the EOS visit
Exclusion criteria	Subject will be excluded from participation if any of the following apply: **1.** Subject has any medical condition that in the opinion of the Investigator makes the subject unsuitable for study participation **2.** Subject is scheduled for emergent surgeries (eg, aortic dissection) **3.** Subject is scheduled for CABG and/or valve surgery and/or ascending aorta aneurysm surgery combined with additional non-emergent cardiac surgeries (eg, congenital heart defects) **4.** Subject is scheduled to undergo transcatheter aortic valve implantation (TAVI) or transcatheter aortic valve replacement (TAVR), or off-pump surgeries or left ventricular assist device (LVAD) implantation **5.** Subject experiences a 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 **6.** Subject has a requirement for any of the following within one week prior to surgery: defibrillator or permanent pacemaker, mechanical ventilation, IABP, LVAD, other forms of mechanical circulatory support (MCS) **7.** Subject has been diagnosed with AKI (as defined by KDIGO criteria) within 3 months prior to surgery **8.** Required cardiopulmonary resuscitation within 14 days prior to cardiac surgery **9.** 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.

	10. Total bilirubin or alanine aminotransferase (ALT) or aspartate aminotransferase (AST) ≥ 2 times the upper limit of normal (ULN) at screening **11.** Subject has a history of solid organ transplantation **12.** Subject has a history of renal replacement therapy (RRT) **13.** Subject has a medical condition which requires active immunosuppressive treatment **14.** Subject has an ongoing chemotherapy or radiation therapy for malignancy that may have an impact on kidney function as assessed by the medical monitor **15.** Subject has received an investigational medicinal product within the last 90 days (or within 5 half-lives of the investigational drug, whichever is longer) **16.** Subject has a known allergy to RMC-035 or one of its constituents, or has previously received RMC-035

Table 2: Other Secondary Endpoints and Exploratory Endpoints

Other Secondary Endpoints	- Post-baseline changes in renal function - SCr and cystatin C (and corresponding eGFR values) at 12, 24,48, and 72 hours, respectively, and at Day 7/discharge, Day 30 and Day 90 - Change from baseline, up to Day 7/discharge, of peak SCr and cystatin C - Time-corrected AUC of cystatin C for Day 1 to Day 4 (72 hours after first dose of IMP) - Need for renal replacement therapy - Dialysis treatment (for any reason) within 72 hours and within 7 days after end of surgery - Dialysis free days from end of surgery to Day 30 and Day 90, respectively - MAKE at Day 30 and Day 90, defined as death, any dialysis, or ≥25% reduction of eGFR compared to baseline Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation (either SCr, cystatin C, or both)[22-24] - AKI Characteristics - AKI within 72 hours after first dose of IMP based on cystatin C and/or UO (AKI of any stage/severity defined as cystatin C ≥1.5 baseline, OR UO <0.5 mL/kg/h for ≥6 hours) - AKI within 7 days after first dose of IMP (based on SCr and/or UO criteria, or cystatin C and/or UO criteria) - AKI persistence, defined as an AKI (KDIGO definition) developing within 72 hours after first dose of IMP and with a duration of ≥72 hours. Persistence will also be assessed per AKI severity stage* - AKI severity stage* within 72 hours and within 7 days after first dose of IMP - *Severity of AKI defined as the following: -Stage 1: SCr 1.5 to 1.9 times baseline within 7 days, OR ≥0.3 mg/dL (≥26.5 µmol/L), OR urine output <0.5 mL/kg/h for 6 to <12 hours -Stage 2: SCr 2.0-2.9 times baseline within 7 days OR urine output <0.5 mL/kg/h for ≥12 hours -Stage 3: SCr 3.0 times baseline within 7 days, OR increase in SCr 4.0 mg/dL (≥353.6 µmol/L), OR initiation

	of renal replacement therapy OR urine output <0.3 mL/kg/h for ≥24 hours OR anuria for ≥12 hours - Post-baseline changes in urine albumin to creatinine ratio (UACR) and urine protein to creatinine ratio (UPCR) at Day 4, Day 30, and Day 90 - Pharmacokinetics of RMC-035 in plasma (AUC and Cmax) - Presence and titers of ADA at Day 1 (pre-surgery), Day 30, and Day 90 - Characteristics of ADA developed at Day 30 and Day 90 with regards to isotype, neutralizing capacity, and cross-reactivity with endogenous alpha-1-microglobulin (A1M)
Exploratory Endpoints	**Post-baseline changes in kidney and cardiac biomarkers** - Kidney biomarkers: Urine kidney injury molecule 1 (KIM-1), neutrophil gelatinase-associated lipocalin (NGAL), tissue inhibitor of metalloproteinase 2 (TIMP2), insulin like growth binding factor protein 7 (IGFBP7), chemokine ligand 14 (CCL-14), interleukin-18 (IL-18), liver fatty acid binding protein (LFABP) and 8-hydroxy-2'-deoxyguanosine (8-OHdG) - Cardiac Biomarkers: Plasma N-terminal-pro-hormone BNP (NT-pro BNP) and cardiac troponin I and T (cTnI, cTnT) **Hospitalization time and discharge facility** - Length of index ICU stay and index hospital stay - Index ICU stay (in Days) defined as the duration of stay in the ICU Immediately following surgery or recovery room post-surgery until ICU discharge - Index hospital stay (in Days) is defined as the duration of stay in the hospital from the day of surgery to hospital discharge for the index surgery - Nature of subject discharge facility (eg, home, skilled nursing facility, Rehabilitation center) **Health-related Quality of Life assessments** - Change from baseline to Day 90 in the following Patient Reported Outcomes (PROs): - MOS 36-Item Short Form Survey Instrument (SF-36) - European Quality of Life 5 Domain 5-Level Score (EQ-5D-5L)

Table 3: Schedule of Assessments – Complete Study Protocol

Assessments	Screening	Treatment							Follow-Up			
Visit Number	**1** [h]	**2 Day of Surgery**					**3**	**4 (EOT)**	**5** [u]	**6 (discharge)** [v]	**7** [w]	**8 (EoS)** [w, x]
Visit Day	**-30 to -1**	**1**					**2 (24h)**	**3 (48h)**	**4 (72h)**	**7**	**30**	**90**
Allowed visit window (days)	**±0**	**±0**					**±0**	**±0**	**±0** [q]	**±2**	**±3**	**±7**
Visit hour (h)		pre	0h	1h	2h	6h [y]	12 h [y]					
Informed consent	x											
Inclusion/exclusion criteria	x	x										
Medical history	x											
Demographics	x											
Weight and height [a]	x						x	x	x			
Physical examination [b]	x								x			
Pregnancy test (WOCBP only) [c]	x	x										
Hematology lab [d]	x						x	x	x			
Clinical chemistry lab [d]	x						x	x	x			
Liver function lab [d]	x						x	x	x			
Serum creatinine (SCr) [e]	x	x					x	x	x	x	x	x
Serum Cystatin C [f]	x	x					x	x	x	x	x	x
UACR and UPCR [g]	x	x							x		x	x
Urinalysis		x							x			
Randomization [h]	x											
Record renal replacement therapy								x	x		x	x
IMP administration [i]			x [j]			x [k]	x [l]	x [l]	x [l]			
Plasma PK sampling		x		x	x			x, x, x [m]	x, x, x [m]			
12-lead electrocardiogram	x	x						x	x	x		
Vital signs [n]	x	x						x	x	x		
Surgery assessments [o]			←				→					

Discharge from ICU [p]							←					→
Urine output [q]		←										→
Urine sampling for biomarkers [r]		x				x		x	x			
Plasma/serum sampling for biomarkers [r]		x						x		x		
HRQoL Assessments [s]	x											x
ADA assessment		x									x	x
Concomitant medication recording [t]	←											→
AE recording				←								→
SAE recording		←										→

Abbreviations: ADA = anti-drug antibody; AE = adverse event; AKI = acute kidney injury; EOS = End of Study; EOT = End-of-Treatment; EQ-5D-5L = European Quality of Life 5 Domain 5-Level Score; HRQoL = health related quality of life; ICU = intensive care unit; IMP = investigational medicinal product; PK = pharmacokinetic; PRO = patient reported outcome; SAE = serious adverse event; SCr = serum creatinine; WOCBP = woman of childbearing potential; UACR = urine albumin to creatinine ratio; UAPR = urine albumin to protein ratio

a. Height only measured at screening (Visit 1). Weight during ICU stay only required if possible.

b. The initial physical examination performed at screening should be comprehensive; all other physical examinations may be abbreviated and symptom driven.

c. A serum pregnancy test completed during the screening period within 48 hours prior to surgery does not need to be repeated on the day of surgery. If the serum pregnancy test occurs more than 48 hours prior to the date of surgery, a serum or urine pregnancy test will also be performed on Day 1 prior to surgery.

d. Hematology Labs: Hematocrit, Hemoglobin (Hb), Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration, Red cell distribution width, Red blood cells, Platelets, Leucocytes (including Neutrophils, Monocytes, Lymphocytes, Eosinophils, Basophils)
Clinical Chemistry Labs: Albumin, Calcium, Chloride, Serum creatinine (SCr), C-reactive protein (CRP), Sodium, estimated glomerular filtration rate (eGFR), Magnesium, Phosphate, Potassium, Blood urea nitrogen (BUN), Uric acid, Glucose
Liver Function Labs: Alanine aminotransferase (ALAT), Alkaline phosphatase (ALP), Aspartate aminotransferase (AST), Bilirubin (total and conjugated), Gamma glutamyltransferase (GGT)

e. The screening sample for SCr must be collected on Day -1 (or day of surgery, see **footnote h**) and will be analyzed locally (to evaluate eligibility and determine correct start dose of RMC-035 according to renal function) and centrally (as baseline for endpoint assessment). All SCr samples collected during hospital stay will be analyzed both locally (to support AKI evaluation) and in a central lab (for the purpose of endpoint assessments). Day 30 and 90 samples will be analyzed centrally.

f. Cystatin C samples will be collected and analyzed in a central lab only for the purpose of endpoint assessments

g. UACR: screening sample will be collected as a spot urine sample and analyzed locally to evaluate albuminuria as an eligibility criterion (in the absence of historical albuminuria data within 3 months prior to randomization). UACR and UPCR: In-hospital samples (Day 1, Visit 2 and Day 4, Visit 5) will be collected either as a First Morning Void (FMV) sample or drawn directly from a Foley catheter and analyzed in a central lab only. Follow-up samples (Day 30, Visit 7 and Day 90, Visit 8) will be collected as FMV samples as possible and analyzed in a central lab only.

h. Randomization must occur on Day -1, ie the day before surgery is intended. All screening assessments may be performed on Day 1 prior to surgery, including randomization. These assessments must be completed prior to any pre-surgical activities, such as administration of fluids or medications, including anesthesia.

i. All 5 doses to be calculated using the same weight measurement that is used for randomization / stratification. IMP will be permanently discontinued in subjects developing AKI stage 2 or higher as per KDIGO guidelines

j. IV infusion over 60 minutes, first infusion should start approximately 10 minutes before expected onset of CPB (time point 0 is defined as start of IMP administration)

k. IV infusion over 60 minutes at 6 h (±30 min) after the start of first infusion

l. IV infusion over 30 minutes at 12 h, 24 h and 48 h (±30 min) after the start of first infusion

m. PK sampling at Day 2 and 3 should occur 30 min (±5 min) and 90 min (±15 min) from start of IMP infusion

Plasma PK Sampling Study Day	Time Window	**Predose** ≤30 min	**30 min** ±5 min	**1 h** ±5 min	**90 min** ±15 min	**2 h** ±15 min
Day 1	Start of Infusion 1 (t=0 h)	x		x		x
Day 2	Start of Infusion 4 (t=24 h)	x	x		x	
Day 3	Start of Infusion 5 (t=48 h)	x	x		x	

n. Vital signs: body temperature, blood pressure, heart rate, respiratory rate, SpO2

o. Data points to collect are type of CPB pump (pulsatile or non-pulsatile, if applicable) and duration of CPB (exact time of initiation and end of CPB), duration of surgery (beginning of surgery defined as exact time of initial skin incision, end of surgery defined as exact time of skin closure), blood loss volume, administration of any fluids during surgery (blood products [red blood cells, plasma, cryoprecipitate, platelets, etc.], crystalloids, colloids, and others), target body temperature during CPB and time at temperature range, duration of cross clamp (minutes), number, position, and graft source bypasses performed, length of time with mean arterial pressure <50 mmHg, valve surgery type (replacement or repair), replacement valve origin (bioprosthetic or mechanical), aortic repair type, and time of admission to the ICU.

p. Time of discharge from ICU to hospital ward, another treatment facility or home

q. Only required as long as Foley catheter is in place

r. Urine samples for evaluation of exploratory urinary (kidney) biomarkers and plasma/serum samples for evaluation of cardiac biomarkers will be collected at the intervals described below, respectively:

Biomarker Sampling Time Window (in relation to Start of Infusion 1 (t=0 h))	**Predose** ≤60 min	**6 h** ±30 min	**24 h** ±30 min	**48 h** ±30 min	**72 h** ±30 min
Urine	x	x	x	x	
Plasma/serum	x		x		x

s. PRO HRQoL assessment: SF 36 and EQ-5D-5L. PRO HRQoL assessments to be performed as early as possible in the screening period.

t. Medications taken within 30 days prior to the day surgery is intended are to be collected. Use of contrast agent within 72 hours prior to the day surgery is intended should be documented as a prior/concomitant medication. When possible, type and quantity of contrast agent should be recorded.

u. Visit 5 must occur at 72 hours from start of first infusion of IMP, with a scheduling window of +/-2 hours.

v. Visit 6 and all associated assessments should occur on the day of hospital discharge. In case subject is discharged on Day 4, discharge (Visit 6) assessments performed prior to discharge on that day are acceptable.

w. Visit may be performed by qualified and trained study staff at the subject's home or other suitable location, where appropriate

x. In case of subject withdrawal, subject should be encouraged to undergo all EOS assessments as an Early Termination visit.

y. Assessments must be performed prior to IMP administration

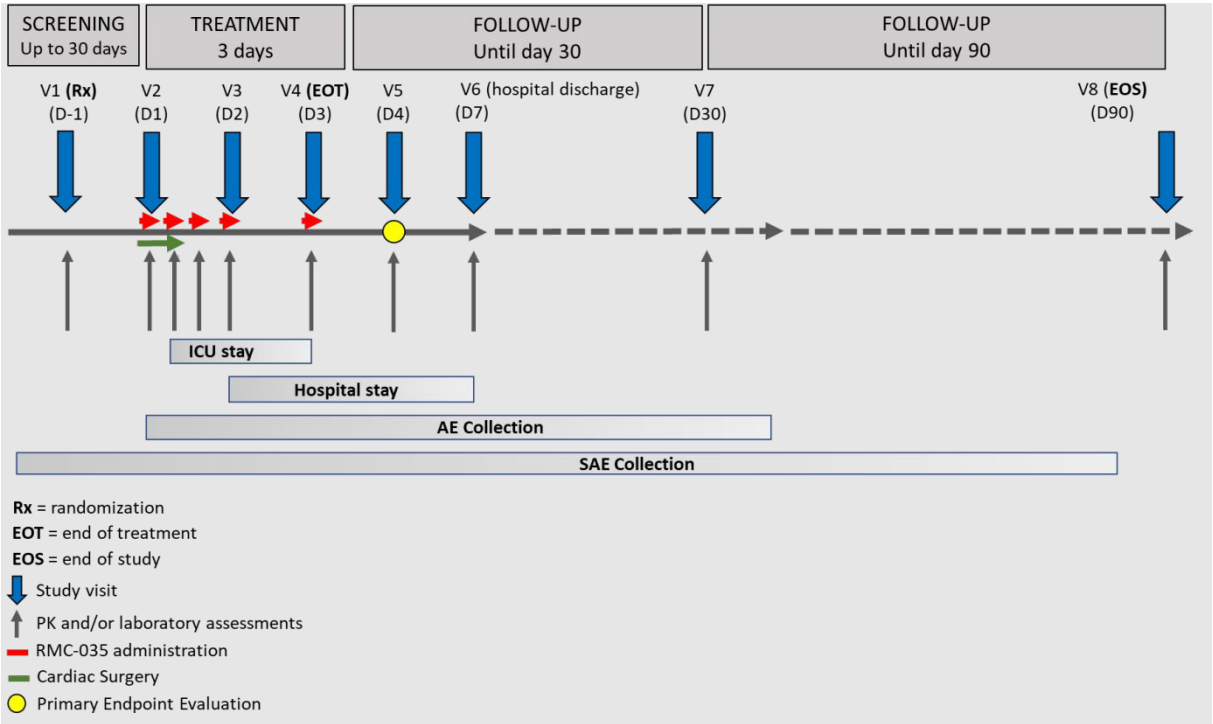

Figure 1: Study Flow Chart

CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	1
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	2
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	4,5
	2b	Specific objectives or hypotheses	6
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	6-11
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	6-11
Participants	4a	Eligibility criteria for participants	6,Table 1, Table 3
	4b	Settings and locations where the data were collected	1
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	8,9
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	6,7,Table 2
	6b	Any changes to trial outcomes after the trial commenced, with reasons	-
Sample size	7a	How sample size was determined	9,10
	7b	When applicable, explanation of any interim analyses and stopping guidelines	9,10
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	8
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	8
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	8
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	8

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Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those assessing outcomes) and how	7,8
	11b	If relevant, description of the similarity of interventions	-
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	11-12
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	10
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	-
	13b	For each group, losses and exclusions after randomisation, together with reasons	-
Recruitment	14a	Dates defining the periods of recruitment and follow-up	-
	14b	Why the trial ended or was stopped	-
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	-
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	-
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	-
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	-
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	-
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	-
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	3
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	3
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	-
Other information			
Registration	23	Registration number and name of trial registry	2
Protocol	24	Where the full trial protocol can be accessed, if available	-
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	16

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.

Patientenschutz | Forschungsfreiheit

ÄRZTEKAMMER WESTFALEN-LIPPE

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9. Februar 2022 FH

Aktenzeichen (bitte immer mit angeben):	**2021-778-f-A**
Sponsor:	Guard Therapeutics International AB, Nybrogatan 34, 114 39 Stockholm
EudraCT-Nummer:	2021-004040-19
Titel der Studie:	A Phase 2, Randomized, Placebo-Controlled, Double-Blind, Adaptive, Parallel Group Clinical Study to Evaluate the Efficacy and Safety of RMC-035 in Subjects at High Risk for Acute Kidney Injury Following Open-Chest Cardiac Surgery
Hier:	01_Neuantrag als zuständige EK, Antrag vom 03.12.2021

Sehr geehrter Herr Dr. Stetter,

für die oben genannte klinische Prüfung haben Sie die zustimmende Bewertung beantragt. Die Ethik-Kommission hat über Ihren Antrag beraten, dabei auch ergänzende/überarbeitete Unterlagen berücksichtigt und beschlossen:

Die klinische Prüfung wird zustimmend bewertet.

Die Bewertung gilt für die klinische Prüfung, wie sie sich auf Grundlage der in Anhang 1 genannten Unterlagen darstellt, sowie für die in Anhang 2 aufgeführten Prüferinnen/Prüfer, Stellvertreterinnen/Stellvertreter und Prüfstellen.

Für die folgenden Prüferinnen/Prüfer, Stellvertreterinnen/Stellvertreter und Prüfstellen liegen noch nicht alle angeforderten Unterlagen vor. Die Bewertung ist noch nicht abgeschlossen:

Prüfstelle: Herzzentrum Dresden GmbH, Fetscherstraße 76, 01307 **Dresden**
Prüfer: Prof. Dr. Klaus Matschke
Stellvertreter: Dr. Konstantin Alexiou

Prüfstelle: Westdeutsches Herzzentrum Essen, Hufelandstraße 55, 45122 **Essen**
Prüfer: Prof. Dr. Matthias Thielmann
Stellvertreter: Dr. Sharaf-Eldin Shehada

Prüfstelle: Universitätsklinikum Halle-Wittenberg, Ernst-Grube-Str. 40, 06120 **Halle**
Prüfer: Prof. Dr. Gabor Szabo
Stellvertreter: Dr. Gabor Veres

Für die Entscheidung der Ethik-Kommission erhebt die Ärztekammer Westfalen-Lippe Gebühren nach Maßgabe einer Verwaltungsgebührenordnung. Darüber hinaus erhält ein gesonderter Bescheid.

Begründung:

Die Bewertung durch die Ethik-Kommission ergeht auf Grundlage von §§ 40 bis 42 Arzneimittelgesetz (AMG), §§ 7, 8 GCP-Verordnung. Versagungsgründe nach § 42 Abs. 1 S. 7 AMG liegen nicht vor.

Allgemeine Hinweise:

An der Beratung und Beschlussfassung haben die in Anhang 3 aufgeführten Mitglieder der Ethik-Kommission teilgenommen.

Die Ethik-Kommission weist darauf hin, dass unabhängig von der vorliegenden Bewertung die medizinische, ethische und rechtliche Verantwortung für die Durchführung einer klinischen Prüfung beim Sponsor und bei allen an der Prüfung teilnehmenden Prüferinnen/Prüfern verbleibt.

Die Ethik-Kommission der Ärztekammer Westfalen-Lippe und der Westfälischen Wilhelms-Universität Münster ist organisiert und arbeitet gemäß den gesetzlichen Bestimmungen und den GCP-Leitlinien der ICH.

Mit freundlichen Grüßen

Univ.-Prof. Dr. med. Wolfgang E. Berdel
Vorsitzender der Ethik-Kommission

BMJ Open

Study protocol of a Phase 2, Randomized, Placebo-Controlled, Double-Blind, Adaptive, Parallel Group Clinical Study to Evaluate the Efficacy and Safety of Recombinant alpha-1-Microglobulin in Subjects at High Risk for Acute Kidney Injury Following Open-Chest Cardiac Surgery (AKITA Trial)

Journal:	*BMJ Open*
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Study protocol of a Phase 2, Randomized, Placebo-Controlled, Double-Blind, Adaptive, Parallel Group Clinical Study to Evaluate the Efficacy and Safety of Recombinant alpha-1-Microglobulin in Subjects at High Risk for Acute Kidney Injury Following Open-Chest Cardiac Surgery (AKITA Trial)

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Abstract

Introduction

Acute kidney injury (AKI) is a common complication after cardiac surgery and is associated with adverse short- and long-term outcomes. Alpha-1-microglobulin (A1M) is a circulating glycoprotein with antioxidant-, heme-binding and mitochondrial-protective mechanisms. RMC-035 is a modified, more soluble, variant of A1M and has been proposed as a novel targeted therapeutic protein to prevent cardiac surgery-associated AKI (CS-AKI). RMC-035 was considered safe and generally well tolerated when evaluated in four clinical Phase 1 studies.

Methods and Analysis

This is a Phase 2, randomized, double-blind, adaptive design, parallel group clinical study that evaluates RMC-035 compared to placebo in approximately 268 cardiac-surgical patients at high risk for CS-AKI. RMC-035 is administered as an IV infusion. In total, five doses will be given. Dosing is based on pre-surgery eGFR, and will be either 1.3 or 0.65 mg/kg.

The primary study objective is to evaluate whether RMC-035 reduces the incidence of post-operative AKI, and key secondary objectives are to evaluate whether RMC-035 improves post-operative renal function compared to placebo. A blinded interim analysis with potential sample size re-assessment is planned once 134 randomized subjects have completed dosing. An independent Data Monitoring Committee will evaluate safety and efficacy data at pre-specified intervals throughout the trial. The study is a global multi-center study at approximately 30 sites.

Ethics and dissemination

The trial was approved by the joint ethics committee of the physician chamber Westfalen-Lippe and the University of Münster (code “2021-778-f-A”) and subsequently approved by the responsible ethics committees/relevant institutional review boards for the participating sites. The study is conducted in accordance with Good Clinical Practice, the Declaration of Helsinki, and other applicable regulations. Results of this study will be published in a peer-reviewed scientific journal.

Trial registration number

EudraCT Number: 2021-004040-19

ClinicalTrials.gov Identifier: NCT05126303

Keywords

Acute renal failure, adult intensive & critical care, A1M, cardiac surgery-associated AKI, CS-AKI.

Article Summary

Strengths and limitations of this study

- This is a placebo-controlled Phase 2 trial powered to evaluate the prevention of AKI in cardiac surgery patients at increased risk for AKI. Results may also allow for evaluation of other clinically relevant outcomes such as post-operative renal function up to 90 days after surgery or health-related quality of life.
- The trial was designed by a group of global experts in AKI and cardiac surgery, with input from or review by the US Food and Drug Administration and several national medicinal regulatory authorities in Europe and North America.
- This trial uses an adaptive trial design whilst preserving data integrity based on a blinded interim analysis, allowing for re-assessment of statistical assumptions, e.g. AKI event rate, with the possibility to increase the number of patients to be recruited.
- Trial subjects will be recruited at multiple centers in Europe and North America that are considered to reflect standard-of-care in cardiac surgery.
- Limitation: The trial is not powered to show differences in less frequently occurring longer term outcomes such as major adverse kidney events (MAKE).

Introduction

Globally, an estimated 2 million cardiovascular procedures per year are performed.[1] Acute kidney injury (AKI) is considered an important determinant of mortality in patients undergoing cardiovascular surgery.[2] [3] Various epidemiological studies show that cardiac surgery-associated AKI (CS-AKI) occurs in up to 30% of cardiac surgery patients [4] and is associated with an increased morbidity as well as short- and long-term mortality.[5-8] The short-term and long-term mortality rate are 10.7% and 30% respectively and increase along with severity of stages.[6] Heart failure, chronic hyperglycemia, anemia, obesity, preoperative exposure to nephrotoxic drugs or contrast media, inflammation, proteinuria, and pre-existing kidney disease were systematically reviewed and were found to be associated with an increased risk of postoperative CS-AKI.[9]

In clinical studies evaluating potential treatments to prevent CS-AKI in high-risk groups, event rates of AKI up to 65.5% in a high-risk population [10] and 71.7% in patients with a positive biomarker test (that identifies patients at very high risk for AKI) shortly after surgery [11] have been reported. Recent studies on CS-AKI showed that patient enrichment based on the combination of the complexity of surgery and co-morbidities resulted in AKI rates between 28 and 34.2%.[12 13]

Current treatment strategies and clinical management of CS-AKI are mainly supportive, including optimal fluid management and maintenance of hemodynamic stability.[14] At present, no specific therapeutic interventions are available for prevention or treatment of CS-AKI. This is an area of large unmet clinical need, where patients who develop CS-AKI are at risk for additional severe complications, including need for renal replacement therapy (RRT), development of chronic kidney disease (CKD) and mortality. Thus, a single AKI episode after cardiac surgery can lead to a significant burden of comorbidities, prolonged hospital stays, poor quality of life and high long-term costs.

Recombinant alpha-1-microglobulin (RMC-035)

The investigational medicinal product (IMP), RMC-035, contains the pharmacologically active protein RMC-035, which is a recombinant and modified variant of endogenous human alpha-1-microglobulin (A1M). It has four distinct molecular mechanisms, including heme binding, reductase activity, radical scavenging, and mitochondrial binding/protection.[15] Employing several antioxidative mechanisms, it protects cells and tissues from various forms of cell damage caused by oxidative stress, including free radicals and reactive oxygen species (ROS). [16-19]

RMC-035 has been evaluated in four Phase 1 clinical studies: single ascending dose and multiple ascending dose studies in healthy subjects, a renal impairment study, and a phase 1b

pharmacokinetic and safety study in cardiac surgery patients. Available clinical data indicate that RMC-035 is safe and generally well tolerated across study populations and support its continued development in CS-AKI.

The pharmacological characteristics of RMC-035 have are appropriate for the chosen indication of CS-AKI as it targetsmultiple critical disease pathways of CS-AKI, including ischemia-reperfusion injury (IRI), heme toxicity and mitochondrial dysfunction. Furthermore, RMC-035 is rapidly distributed to kidney proximal tubular cells (via glomerular filtration), resulting in high drug exposure in the cells where the primary injury occurs in AKI. It is administered as intravenous (IV) infusion with short systemic half-life which is ideal for hospitalized patients requiring specific renal protection.

Trial objectives

This is a Phase 2, randomized, placebo-controlled, double-blind, adaptive design, parallel group clinical study to evaluate the efficacy and safety of RMC-035 in subjects at high risk for AKI following open-chest cardiac surgery. The study is being conducted to provide proof-of-concept efficacy data and to guide the design of a subsequent pivotal study in this patient population. Based on an integrated assessment of available non-clinical and clinical data, RMC-035 is considered to be well tolerated and is expected to provide targeted renal protection in this patient population, with the aim to prevent or ameliorate peri-operative and post-surgery renal injury.

Primary Objectives are to evaluate RMC-035 for the following outcomes:

• Prevention of AKI (Kidney Disease Improving Global Outcomes [KDIGO] definition [20]) in subjects undergoing cardiac surgery with additional risk factors for developing CS-AKI

• Safety and tolerability

Key secondary objectives are to evaluate RMC-035 for the following outcomes:

• Prevention of post-operative decline (within 72 hours) in renal function

• Reduction of post-operative AKI duration

Other secondary objectives are to evaluate RMC-035 for the following outcomes:

• Preserving post-surgery renal function up to Day 90

• Prevention of post-operative dialysis up to Day 90

• Prevention of major adverse kidney events (MAKE) at Days 30 and 90, respectively

• Prevention of AKI within 72 hours (based on cystatin C and/or Urine Output [UO])

- Persistence and severity of AKI within 72 hours (based on serum creatinine [SCr] and/or UO or cystatin C and/or UO)
- Prevention, persistence, and severity of AKI within 7 days (based on SCr and/or UO or cystatin C and/or UO)
- Reduction of post-operative albuminuria and proteinuria up to Day 90
- Pharmacokinetics of RMC-035
- Identification and characterization of anti-drug-antibodies (ADA)

Exploratory objectives are to evaluate:

- Post-baseline changes in kidney and cardiac biomarkers
- Length of post-operative stay in Intensive Care Unit (ICU) and overall hospitalization time
- Health-related Quality of Life

Methods and analysis

Patients

The target patient population is adults undergoing open-chest cardiac surgery, i.e. CABG and/or valve surgery and/or aorta surgery with additional predisposing risk factors for CS-AKI. Subjects planned to undergo cardiac surgery will be systematically screened for eligibility. Informed consent will be obtained by study site's principal or sub-investigators in all eligible patients before randomization and surgery. Inclusion and exclusion criteria are described in Table 1.

Table 1: Inclusion and Exclusion criteria

Inclusion criteria	A subject is eligible for the study if all of the following apply: 1. Institutional Review Board/ International Ethics Committee approved Informed Consent obtained 2. Ability to understand and comply with the study requirements and able to provide written informed consent 3. Age ≥18 and <85 years 4. Estimated glomerular filtration rate (eGFR) is ≥30 mL/min/1.73 m2 5. Subject is scheduled for non-emergent coronary artery bypass grafting (CABG) surgery and/or valve surgery and/or ascending aorta aneurysm surgery with use of cardiopulmonary bypass (CPB), and AKI risk factors are present at screening

	6. Female subject is not of child-bearing potential, or agreeing not to become pregnant 7. Female subject must not be breastfeeding 8. Female subject must not donate ova 9. Male subject and their female spouse/partner(s) who are of childbearing potential must be using a highly effective form of birth control 10. Male subjects must not donate sperm 11. Subject agrees not to participate in another interventional study
Exclusion criteria	Subject will be excluded from participation if any of the following apply: 1. Medical condition that makes the subject unsuitable for study participation 2. Scheduled for emergent surgeries (eg, aortic dissection) 3. Scheduled for CABG and/or valve surgery and/or ascending aorta aneurysm surgery combined with additional non-emergent cardiac surgeries (eg, congenital heart defects) 4. Scheduled to undergo transcatheter aortic valve implantation (TAVI) or transcatheter aortic valve replacement (TAVR), or off-pump surgeries or left ventricular assist device (LVAD) implantation 5. Experiences a cardiogenic shock or hemodynamic instability which require inotropes or vasopressors or other mechanical devices within 24 hours prior to surgery 6. Requirement for defibrillator or permanent pacemaker, mechanical ventilation, intraaortic balloon pumping (IABP), LVAD, or other forms of mechanical circulatory support (MCS) 7. Diagnosed with AKI (as defined by KDIGO criteria) within 3 months prior to surgery 8. Required cardiopulmonary resuscitation within 14 days prior to cardiac surgery 9. Ongoing sepsis or an untreated diagnosed clinically significant infection (viral or bacterial) 10. Total bilirubin or alanine aminotransferase (ALT) or aspartate aminotransferase (AST) ≥ 2 times the upper limit of normal (ULN)

	11. History of solid organ transplantation 12. History of renal replacement therapy (RRT) 13. Medical condition which requires active immunosuppressive treatment 14. Ongoing chemotherapy or radiation therapy for malignancy that may have an impact on kidney function 15. Received an investigational medicinal product within the last 90 days (or within 5 half-lives of the investigational drug, whichever is longer) 16. Subject has a known allergy to RMC-035 or one of its constituents, or has previously received RMC-035

Main outcomes

Primary Endpoints

Primary Efficacy Endpoint

Proportion of patients developing AKI within 72 hours after first dose of IMP, based on SCr and/or UO (AKI of any stage/severity according to KDIGO definition, ie, SCr ≥1.5 times baseline, or increase of SCr of ≥0.3 mg/dL [≥26.5 µmol/L], or UO <0.5 mL/kg/h for ≥6 hours).

Primary Safety Endpoint

Nature, frequency and severity of treatment-emergent adverse events (TEAEs; defined as any AE which occur within 72 hours after last IMP administration).

Secondary Endpoints

Key Secondary Endpoints

Time-corrected area under the curve (AUC) of SCr for Day 1 to Day 4 (72 hours after first dose of IMP)

Duration of AKI defined as the number of days meeting the definition of AKI (KDIGO definition) starting within 72 hours after first dose of IMP until resolution

For other Secondary Endpoints and Exploratory Endpoints, please see Table 2.

Table 2: Other Secondary Endpoints and Exploratory Endpoints

Other Secondary Endpoints	- Post-baseline changes in renal function - SCr and cystatin C (and corresponding eGFR values) at 12, 24,48, and 72 hours, respectively, and at Day 7/discharge, Day 30 and Day 90 - Change from baseline, up to Day 7/discharge, of peak SCr and cystatin C - Time-corrected AUC of cystatin C for Day 1 to Day 4 (72 hours after first dose of IMP) - Need for renal replacement therapy - Dialysis treatment (for any reason) within 72 hours and within 7 days after end of surgery - Dialysis free days from end of surgery to Day 30 and Day 90, respectively - MAKE at Day 30 and Day 90, defined as death, any dialysis, or ≥25% reduction of eGFR compared to baseline Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation (either SCr, cystatin C, or both)[21-23] - AKI Characteristics - AKI within 72 hours after first dose of IMP based on cystatin C and/or UO (AKI of any stage/severity defined as cystatin C ≥1.5 baseline, OR UO <0.5 mL/kg/h for ≥6 hours) - AKI within 7 days after first dose of IMP (based on SCr and/or UO criteria, or cystatin C and/or UO criteria) - AKI persistence, defined as an AKI (KDIGO definition) developing within 72 hours after first dose of IMP and with a duration of ≥72 hours. Persistence will also be assessed per AKI severity stage* - AKI severity stage* within 72 hours and within 7 days after first dose of IMP - *Severity of AKI defined as the following: -Stage 1: SCr 1.5 to 1.9 times baseline within 7 days, OR ≥0.3 mg/dL (≥26.5 µmol/L), OR urine output <0.5 mL/kg/h for 6 to <12 hours -Stage 2: SCr 2.0-2.9 times baseline within 7 days OR urine output <0.5 mL/kg/h for ≥12 hours -Stage 3: SCr 3.0 times baseline within 7 days, OR increase in SCr 4.0 mg/dL (≥353.6 µmol/L), OR initiation of renal replacement therapy OR urine output <0.3

	mL/kg/h for ≥24 hours OR anuria for ≥12 hours - Post-baseline changes in urine albumin to creatinine ratio (UACR) and urine protein to creatinine ratio (UPCR) at Day 4, Day 30, and Day 90 - Pharmacokinetics of RMC-035 in plasma (AUC and Cmax) - Presence and titers of ADA at Day 1 (pre-surgery), Day 30, and Day 90 - Characteristics of ADA developed at Day 30 and Day 90 with regards to isotype, neutralizing capacity, and cross-reactivity with endogenous alpha-1-microglobulin (A1M)
Exploratory Endpoints	**Post-baseline changes in kidney and cardiac biomarkers** - Kidney biomarkers: Urine kidney injury molecule 1 (KIM-1), neutrophil gelatinase-associated lipocalin (NGAL), tissue inhibitor of metalloproteinase 2 (TIMP2), insulin like growth binding factor protein 7 (IGFBP7), chemokine ligand 14 (CCL-14), interleukin-18 (IL-18), liver fatty acid binding protein (LFABP) and 8-hydroxy-2'-deoxyguanosine (8-OHdG) - Cardiac Biomarkers: Plasma N-terminal-pro-hormone BNP (NT-pro BNP) and cardiac troponin I and T (cTnI, cTnT) **Hospitalization time and discharge facility** - Length of index ICU stay and index hospital stay - Index ICU stay (in Days) defined as the duration of stay in the ICU Immediately following surgery or recovery room post-surgery until ICU discharge - Index hospital stay (in Days) is defined as the duration of stay in the hospital from the day of surgery to hospital discharge for the index surgery - Nature of subject discharge facility (eg, home, skilled nursing facility, Rehabilitation center) **Health-related Quality of Life assessments** - Change from baseline to Day 90 in the following Patient Reported Outcomes (PROs): - MOS 36-Item Short Form Survey Instrument (SF-36) - European Quality of Life 5 Domain 5-Level Score (EQ-5D-5L)

Trial oversight

A scientific advisory board of experts in anesthesiology and cardiac surgery has been established by the sponsor to facilitate design of the trial, provide medical scientific leadership and make trial-specific recommendations to the sponsor. Likewise, an independent unblinded data monitoring committee (DMC) consisting of experts in acute kidney injury and a statistician will perform review of safety findings at regular intervals during the study. The working procedures of the DMC are defined in a DMC charter; their safety evaluation will be based on all available accumulated safety data. The DMC will also be responsible for recommendation on the study conduct following the interim analysis (IA) based upon predefined unblinded statistical output. Communication and recommendations by the DMC will be blinded. Completeness and accuracy of the data will be monitored by a Clinical Research Organization which performs regular monitoring visits.

Patient and public involvement statement

Reflecting the acute and unpredictable nature of CS-AKI there are no specific patient advocacy groups at present; closest are organizations that represent kidney diseases after cardiac surgery in general and, although they are aware of our program, they were not specifically consulted on the design of this study.

Trial design

Overall study design

This is a Phase 2, multi-center, randomized, double-blind, placebo-controlled, adaptive design, parallel group trial including patients undergoing open-chest cardiac surgery. The study consists of a screening period of up to 30 days before surgery, five in-hospital visits during the anticipated post-operative hospitalization period, and two additional follow-up visits at Day 30 and Day 90, respectively. A study flow chart is shown in Figure 1 and the schedule of study specific assessments is presented in Table 3. The study reporting was assessed following SPIRIT guidelines and is presented in the SPIRIT checklist in the supplementary material.

Table 3: Schedule of Assessments – Complete Study Protocol

Assessments	**Screening**	**Treatment**							**Follow-Up**			
Visit Number	**1** [h]	**2 Day of Surgery**					**3**	**4 (EOT)**	**5** [u]	**6 (discharge)** [v]	**7** [w]	**8 (EoS)** [w, x]
Visit Day	**-30 to -1**	**1**					**2 (24h)**	**3 (48h)**	**4 (72h)**	**7**	**30**	**90**
Allowed visit window (days)	**±0**	**±0**					**±0**	**±0**	**±0** [q]	**±2**	**±3**	**±7**
Visit hour (h)		pre	0h	1h	2h	6h [y]	12 h [y]					
Informed consent	x											
Inclusion/exclusion criteria	x	x										
Medical history	x											
Demographics	x											
Weight and height [a]	x						x	x	x			
Physical examination [b]	x								x			
Pregnancy test (WOCBP only) [c]	x	x										
Hematology lab [d]	x						x	x	x			
Clinical chemistry lab [d]	x						x	x	x			
Liver function lab [d]	x						x	x	x			
Serum creatinine (SCr) [e]	x	x					x	x	x	x	x	x
Serum Cystatin C [f]	x	x					x	x	x	x	x	x
UACR and UPCR [g]	x	x							x		x	x
Urinalysis		x							x			
Randomization [h]	x											
Record renal replacement therapy							x	x	x		x	x
IMP administration [i]			x [j]			x [k]	x [l]	x [l]	x [l]			
Plasma PK sampling		x		x	x			x, x, x [m]	x, x, x [m]			
12-lead electrocardiogram	x	x						x	x	x		
Vital signs [n]	x	x						x	x	x		
Surgery assessments [o]			←			→						
Discharge from ICU [p]							←					→

Urine output [q]		←										→
Urine sampling for biomarkers [r]		x				x		x	x				
Plasma/serum sampling for biomarkers [r]		x						x		x			
HRQoL Assessments [s]	x												x
ADA assessment		x										x	x
Concomitant medication recording [t]	←												→
AE recording				←									→
SAE recording		←											→

Abbreviations: ADA = anti-drug antibody; AE = adverse event; AKI = acute kidney injury; EOS = End of Study; EOT = End-of-Treatment; EQ-5D-5L = European Quality of Life 5 Domain 5-Level Score; HRQoL = health related quality of life; ICU = intensive care unit; IMP = investigational medicinal product; PK = pharmacokinetic; PRO = patient reported outcome; SAE = serious adverse event; SCr = serum creatinine; WOCBP = woman of childbearing potential; UACR = urine albumin to creatinine ratio; UAPR = urine albumin to protein ratio

a. Height only measured at screening (Visit 1). Weight during ICU stay only required if possible.

b. The initial physical examination performed at screening should be comprehensive; all other physical examinations may be abbreviated and symptom driven.

c. A serum pregnancy test completed during the screening period within 48 hours prior to surgery does not need to be repeated on the day of surgery. If the serum pregnancy test occurs more than 48 hours prior to the date of surgery, a serum or urine pregnancy test will also be performed on Day 1 prior to surgery.

d. Hematology Labs: Hematocrit, Hemoglobin (Hb), Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration, Red cell distribution width, Red blood cells, Platelets, Leucocytes (including Neutrophils, Monocytes, Lymphocytes, Eosinophils, Basophils)
Clinical Chemistry Labs: Albumin, Calcium, Chloride, Serum creatinine (SCr), C-reactive protein (CRP), Sodium, estimated glomerular filtration rate (eGFR), Magnesium, Phosphate, Potassium, Blood urea nitrogen (BUN), Uric acid, Glucose
Liver Function Labs: Alanine aminotransferase (ALAT), Alkaline phosphatase (ALP), Aspartate aminotransferase (AST), Bilirubin (total and conjugated), Gamma glutamyltransferase (GGT)

e. The screening sample for SCr must be collected on Day -1 (or day of surgery, see **footnote h**) and will be analyzed locally (to evaluate eligibility and determine correct start dose of RMC-035 according to renal function) and centrally (as baseline for endpoint assessment). All SCr samples collected during hospital stay will be analyzed both locally (to support AKI evaluation) and in a central lab (for the purpose of endpoint assessments). Day 30 and 90 samples will be analyzed centrally.

f. Cystatin C samples will be collected and analyzed in a central lab only for the purpose of endpoint assessments

g. UACR: screening sample will be collected as a spot urine sample and analyzed locally to evaluate albuminuria as an eligibility criterion (in the absence of historical albuminuria data within 3 months prior to randomization). UACR and UPCR: In-hospital samples (Day 1, Visit 2 and Day 4, Visit 5) will be collected either as a First Morning Void (FMV) sample or drawn directly from a Foley catheter and analyzed in a central lab only. Follow-up samples (Day 30, Visit 7 and Day 90, Visit 8) will be collected as FMV samples as possible and analyzed in a central lab only.

h. Randomization must occur on Day -1, ie the day before surgery is intended. All screening assessments may be performed on Day 1 prior to surgery, including randomization. These assessments must be completed prior to any pre-surgical activities, such as administration of fluids or medications, including anesthesia.

i. All 5 doses to be calculated using the same weight measurement that is used for randomization / stratification. IMP will be permanently discontinued in subjects developing AKI stage 2 or higher as per KDIGO guidelines

j. IV infusion over 60 minutes, first infusion should start approximately 10 minutes before expected onset of CPB (time point 0 is defined as start of IMP administration)

k. IV infusion over 60 minutes at 6 h (±30 min) after the start of first infusion

l. IV infusion over 30 minutes at 12 h, 24 h and 48 h (±30 min) after the start of first infusion

m. PK sampling at Day 2 and 3 should occur 30 min (±5 min) and 90 min (±15 min) from start of IMP infusion

Plasma PK Sampling Study Day	Time Window	**Predose** ≤30 min	**30 min** ±5 min	**1 h** ±5 min	**90 min** ±15 min	**2 h** ±15 min
Day 1	Start of Infusion 1 (t=0 h)	x		x		x
Day 2	Start of Infusion 4 (t=24 h)	x	x		x	
Day 3	Start of Infusion 5 (t=48 h)	x	x		x	

n. Vital signs: body temperature, blood pressure, heart rate, respiratory rate, SpO2

o. Data points to collect are type of CPB pump (pulsatile or non-pulsatile, if applicable) and duration of CPB (exact time of initiation and end of CPB), duration of surgery (beginning of surgery defined as exact time of initial skin incision, end of surgery defined as exact time of skin closure), blood loss volume, administration of any fluids during surgery (blood products [red blood cells, plasma, cryoprecipitate, platelets, etc.], crystalloids, colloids, and others), target body temperature during CPB and time at temperature range, duration of cross clamp (minutes), number, position, and graft source bypasses performed, length of time with mean arterial pressure <50 mmHg, valve surgery type (replacement or repair), replacement valve origin (bioprosthetic or mechanical), aortic repair type, and time of admission to the ICU.

p. Time of discharge from ICU to hospital ward, another treatment facility or home

q. Only required as long as Foley catheter is in place

r. Urine samples for evaluation of exploratory urinary (kidney) biomarkers and plasma/serum samples for evaluation of cardiac biomarkers will be collected at the intervals described below, respectively:

Biomarker Sampling Time Window (in relation to Start of Infusion 1 (t=0 h))	**Predose** ≤60 min	**6 h** ±30 min	**24 h** ±30 min	**48 h** ±30 min	**72 h** ±30 min
Urine	x	x	x	x	
Plasma/serum	x		x		x

s. PRO HRQoL assessment: SF 36 and EQ-5D-5L. PRO HRQoL assessments to be performed as early as possible in the screening period.

t. Medications taken within 30 days prior to the day surgery is intended are to be collected. Use of contrast agent within 72 hours prior to the day surgery is intended should be documented as a prior/concomitant medication. When possible, type and quantity of contrast agent should be recorded.

u. Visit 5 must occur at 72 hours from start of first infusion of IMP, with a scheduling window of +/-2 hours.

v. Visit 6 and all associated assessments should occur on the day of hospital discharge. In case subject is discharged on Day 4, discharge (Visit 6) assessments performed prior to discharge on that day are acceptable.

w. Visit may be performed by qualified and trained study staff at the subject's home or other suitable location, where appropriate

x. In case of subject withdrawal, subject should be encouraged to undergo all EOS assessments as an Early Termination visit.

y. Assessments must be performed prior to IMP administration

Randomization procedure

The trial population consists of patients at high risk for AKI and undergo cardiac surgery with additional predisposing risk factors for CS-AKI (inclusion and exclusion criteria are shown in Table 1). Eligible subjects will be randomized to receive either RMC-035 or placebo in a 1:1 randomization ratio. Both region (EU versus North America) and pre-operative eGFR calculated using the CKD-EPI equation with SCr on local laboratory results (≥60 and <60 mL/min/1.73m^2) will be used as stratification factors to ensure a balanced randomization within these groups. Randomization will be performed by the investigators using the centralized electronic randomization platform. Randomization codes will be subsequently computer-generated and sent to study pharmacists for IMP preparation.

IMP presentation and blinding

The Investigator, study personnel, and subject will be blinded to the identity of the IMP (RMC-035 or placebo). The investigational pharmacist will be responsible for the preparation of IMP for each subject and will be unblinded to the randomization assignment. In the event of a medical emergency requiring knowledge of the treatment assigned to the subject, unblinding can be performed and the treatment code for a given randomized subject will be provided. The time, date, subject number and reason for obtaining any of these codes, and therefore breaking the blind, will be documented in the study file. The treatment code can only be requested by the Investigator or other persons designated as sub-investigators. No subjects or study personnel will be made aware of the treatment given unless a medical emergency necessitates such disclosure. Both IMP and placebo will be presented in 50ml syringes and labelled in a standardized manner. Label information includes study protocol number, subject-ID, syringe number, date and time of IMP preparation, IMP content (RMC-035 or placebo) and sponsor contact information, but no information about randomization group. Both RMC-035 and placebo are transparent, odorless fluids that cannot be differentiated optically or by other sensations. Pharmacokinetic samples taken at the study sites and evaluated in a central laboratory of the sponsor will be used to determine systemic exposure to study drug.

IMP dosing

In total five IV doses of either RMC-035 or placebo will be administered during the study. The first dose will start approximately 10 minutes before cardiopulmonary surgery is initiated (0h), and subsequent doses will be given at 6, 12, 24, and 48 hours later. IMP will be given as a continuous IV infusion over either 60 minutes (first two doses) or 30 minutes for subsequent doses. Due to the pharmacokinetic properties of RMC-035, the predefined dose levels (prior to surgery) are based on renal function (eGFR by CKD-EPI equation with SCr) at screening (Day -1, Visit 1): 1) subjects with eGFR ≥60 mL/min/1.73m^2 will receive 1.3 mg/kg (per dose) for the first and second dose, followed by 0.65 mg/kg (per dose) for the third, fourth and fifth

dose; 2) Subjects with eGFR >30 and <60 mL/min/1.73m^2 will receive 0.65 mg/kg (per dose) for all five doses. The trial drug is provided on top of standard of care of each center.

Permanent discontinuation of IMP

Local laboratory results will be utilized for clinical care and real time evaluation of AKI for purposes of IMP discontinuation. Subjects who meet any of the following IMP discontinuation criteria during the treatment period will be discontinued from IMP and continue study participation and procedures and follow up as per the schedule of assessment until the end of the study visit: 1) Development of stage ≥2 AKI, according to KDIGO definition, 2) Need for RRT, 3) Need for percutaneous or surgical mechanical circulatory support (MCS) or extra-corporeal membrane oxygenator (ECMO), 4) Reporting of a grade 3 (per Common Terminology Criteria for Adverse Events [CTCAE]) or higher adverse event of ISR or IRR, and which is considered to be an immune-mediated reaction

The IMP will be discontinued in case of any of the following abnormal liver chemistry tests in blood: 1) ALT > 3 × ULN combined with total bilirubin > 2 × ULN in the same sample, 2) ALT > 3 × ULN if associated with symptoms (new or worsening) believed to be related to hepatitis (such as fatigue, nausea, vomiting, right upper quadrant pain or tenderness or jaundice) or believed to be related to hypersensitivity (such as fever, rash or eosinophilia), 3) ALT > 3 x ULN if confirmed in a second sample within 24 hours AND in the second sample International Normalized Ratio (INR) is increased to >1.5 x ULN, 4) ALT > 8 x ULN in any individual sample during the treatment period All liver chemistry abnormalities as summarized above need to be followed up regularly (at least every 24 hours) until values have returned to baseline or are considered stable. If IMP is discontinued, the subject will remain in the study to be evaluated until the end of the study Visit (as much as possible) or complete the end of the study visit assessments.

Planned sample size & interim analysis (IA)

A minimum of 268 and a maximum of 348 patients are planned to be randomized at approximately 30 sites predominantly across Europe and North America. The final number of patients randomized will depend on the results of the IA.

There will be one IA during the trial which will be conducted once 50% of the planned randomized subjects have completed visit 6 (Day 5-9). Sample size may be increased to a maximum of 348 randomized subject but will not be decreased. The study may be stopped at the IA for futility or safety reasons. Study eligibility criteria may also be modified.

Safety reporting

Safety is followed up until last study visit at Day 90. Serious adverse events (SAEs) will be reported to the sponsor and other stakeholders as per regulatory requirements (eg, competent authorities and/or IRBs/CECs, when applicable). Non-serious AEs will only be reported up to Day 30 Treatment emergent AEs (TEAEs) are defined as AEs which occur after the initial IMP administration through 72 hours after last IMP administration. Safety reporting will be managed by a clinical research organization in compliance with regulatory requirements of each region of the study.

Statistical Considerations

For the primary endpoint, KDIGO AKI (serum creatinine and urine output) within 72 hours after first dose of IMP, RMC-035 has been assumed to lead to a 30% relative risk reduction vs. placebo. The event rate in the placebo group has been assumed to be 50%.[24] A sample size of 268 subjects randomized leads to a test power of 80% to show statistically significant results at a two-sided significance level (alpha) of 0.10.

The primary endpoint will be analyzed by the Cochran-Mantel-Haenszel estimate of the common relative risk (RMC-035 vs. placebo) across the four stratification groups formed by region and pre-operative eGFR (≥60 and <60 mL/min/1.73m2). In addition, the proportion of subjects with AKI within 72 hours after first dose of IMP and its 90% confidence interval will be calculated for each treatment group. The hypotheses to be tested are as follows: 1) Null hypothesis: the proportion of subjects developing AKI within 72 hours after first dose of IMP is the same for the RMC-035 and the placebo treatment groups ($\mathbf{p}_{\text{RMC-035}} = \mathbf{p}_{\text{placebo}}$), 2) Alternative hypothesis: the proportion of subjects developing AKI within 72 hours after first dose of IMP is different for the RMC-035 and the placebo treatment groups ($\mathbf{p}_{\text{RMC-035}} \neq \mathbf{p}_{\text{placebo}}$).

All secondary endpoints have a supplementary character and will be reported with no strict control of the type I error. Secondary continuous efficacy endpoints that are measures of renal function will be analyzed using robust regression of log-transformed values having sequentially imputed any missing data using multiple imputation. A sensitivity analysis will be performed using a mixed model for repeated measurements (MMRM) if data are approximately normally distributed.

For the key secondary AUC endpoint, the geometric least square means of the time-corrected AUC of SCr for Days 1 to 4 will be obtained for each, RMC-035 and placebo, by transforming the model estimates back to the original scale.

Supportive analyses of renal function will be performed on cystatin C levels (and corresponding eGFR values assessed by Chronic Kidney Disease Epidemiology Collaboration [CKD-EPI] equations with either SCr, cystatin C, or both) and cumulative AUC values up to each timepoint. Secondary binary efficacy endpoints will be analyzed using the same approach as specified above for the primary endpoint.

Further details on the IA and the control of the type I error will be provided in the DMC Charter and the Statistical Analysis Plan (SAP).

Safety Endpoints

Safety analyses will be conducted on the Safety Analysis Set (SAF), which includes all randomized subjects who received at least 1 dose of IMP. To characterize the safety profile, the number and percentage of subjects with TEAEs and AEs per dose group will be tabulated. Descriptive statistics will be provided for laboratory tests (hematology, biochemistry, urinalysis) and vital signs (pulse rate, respiratory rate and blood pressure) by visit and for the changes from baseline to each visit split by treatment group. In addition, the development of anti-drug antibodies after intravenous administration of RMC-035 will be investigated and characterized.

Pharmacokinetics

Descriptive statistics will be presented for plasma concentrations by scheduled sample time. Specific pharmacokinetic parameters will be presented separately. Summaries will be provided by dose group, age group and by renal function prior to surgery.

Sensitivity analyses and secondary endpoints

Secondary continuous efficacy endpoints that are measures of renal function will be analyzed using robust regression of log-transformed values having sequentially imputed any missing data using multiple imputation. A sensitivity analysis will be performed using a mixed model for repeated measurements (MMRM) if data are approximately normally distributed.

For the key secondary AUC endpoint, the geometric least square means of the time-corrected AUC of SCr for Days 1 to 4 will be obtained for each, RMC-035 and placebo, by transforming the model estimates back to the original scale. The relative difference (RMC-035 vs. placebo) and its 90% confidence interval will also be reported.

Supportive analyses of renal function will be performed by using cystatin C levels (and corresponding eGFR values assessed by Chronic Kidney Disease Epidemiology Collaboration [CKD-EPI] equations with either SCr, cystatin C, or both) and cumulative AUC values up to each timepoint.

Secondary binary efficacy endpoints will be analyzed using the same approach as specified above for the primary endpoint.

Additional sensitivity analyses of the primary and secondary endpoints, and evaluation of exploratory endpoints, will be detailed in the SAP.

Ethics and dissemination

This trial will be conducted in accordance with the protocol and consensus ethical principles derived from international guidelines including the Declaration of Helsinki and Council for International Organizations of Medical Sciences (CIOMS) International Ethical Guidelines; applicable International Conference on Harmonization (ICH) GCP Guidelines; and applicable laws and regulations. The protocol, substantial protocol amendments, ICF, IB, and other relevant documents (e.g., any other written information regarding this trial to be provided to the patient or the patient's legal representative) are approved by the Ethics Committee of the Physicians Chamber Westfalen-Lippe and the University of Münster in Germany (file number 2021-778-f-A).

Informed consent will be given by patients before any study relevant procedure is performed. Patients will be assigned a unique patient identification number. Any patient records or datasets that are transferred to the Sponsor will contain this identifier only; patient names and any information which would make the patient identifiable will not be transferred. All laboratory specimens, evaluation forms, reports, and other records will be identified in a manner designed to maintain patient confidentiality.

Irrespective of whether the trial is completed or prematurely terminated, the sponsor will ensure that the trial results will be posted on publicly available clinical trial registries in accordance with their requirements. In addition, results will be presented at international congresses and published in peer-reviewed journals. Participant-level access to data will not be granted to the public.

Conclusions

The ongoing Phase 2 (AKITA) study is specifically designed to answer the primary research question whether the investigational drug RMC-035 can reduce the occurrence of CS-AKI within 3 days after open cardiac surgery. Study results will also provide pertinent information on other clinically relevant endpoints, such as the severity, duration and persistence of AKI, changes in post-operative renal function and major adverse kidney events (MAKE), and will significantly expand the current knowledge of the safety profile of RMC-035. Finally, biomarker analysis may improve the understanding of its mechanism of action, shed light on relevant disease pathways in CS-AKI and facilitate the design of future clinical trials of RMC-035.

Author's contributions

AZ, TEL, and RR co-drafted the manuscript. CDM, NDF, JB, FW, AM, CCDB, JLK, AB, DTE, BdV, NN, MT, AL, ML, TvG and CR participated in trial protocol design, reviewed and edited the manuscript.

Funding statement

This work was supported by Guard Therapeutics.

Competing interest statement

Alexander Zarbock received advisory board consultancy and travel reimbursements from Guard Therapeutics.

Data availability statement: Data obtained during the trial is the property of the study sponsor and will be handled in compliance with data protection policies and regulations.

Word count:

3273 words

Figure legend

Figure 1: study flowchart

Ethics Approval

The study was approved by the Ethics Committee of the Physicians Chamber Westfalen-Lippe and the University of Münster in Germany (file number 2021-778-f-A).

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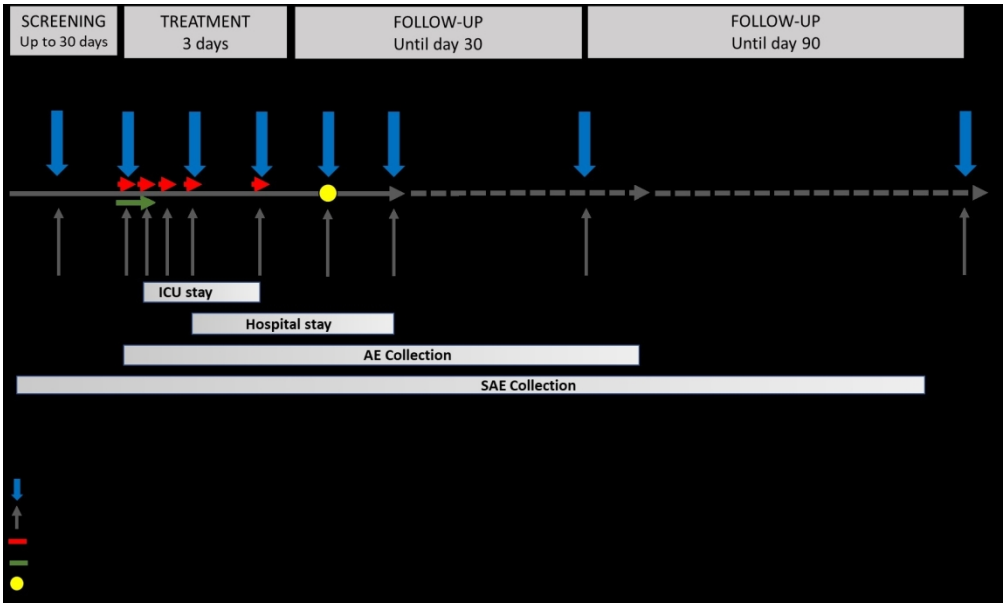

Figure 1: Study flowchart

307x183mm (150 x 150 DPI)

Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

In your methods section, say that you used the SPIRITreporting guidelines, and cite them as:

Chan A-W, Tetzlaff JM, Gøtzsche PC, Altman DG, Mann H, Berlin J, Dickersin K, Hróbjartsson A, Schulz KF, Parulekar WR, Krleža-Jerić K, Laupacis A, Moher D. SPIRIT 2013 Explanation and Elaboration: Guidance for protocols of clinical trials. BMJ. 2013;346:e7586

		Reporting Item	Page Number
Administrative information			
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
11Trial registration	#2a	Trial identifier and registry name. If not yet registered, name of intended registry	2
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	2
Protocol version	#3	Date and version identifier	n/a
Funding	#4	Sources and types of financial, material, and other support	12
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	1
Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	1
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication,	12

		including whether they will have ultimate authority over any of these activities	
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	7
Introduction			
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	4,5
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	n/a
Objectives	#7	Specific objectives or hypotheses	5,6,7
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	8-11
Methods: Participants, interventions, and outcomes			
Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	9
Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	6, Table 1
Interventions:	#11a	Interventions for each group with sufficient detail to	7,8

description		allow replication, including how and when they will be administered	
Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	8,9
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	8,11
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	Table 1
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	5-7
Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	Table 3
Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	9
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	9
Methods: Assignment of interventions (for controlled trials)			
Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of	8

		any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	
Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	8
8Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	8
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	8
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	8
Methods: Data collection, management, and analysis			
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	10,11
Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	7

Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	7
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	10-12
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	11,12
Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	11,12
Methods: Monitoring			
Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	7
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	10
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	10
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent	10

		from investigators and the sponsor	
Ethics and dissemination			
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	2,12
Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	n/a
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	6,12
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	n/a
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	7,12
Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	13
Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	13
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	n/a
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	12

Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	n/a
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	12
Appendices			
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	Supplementary material
Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	n/a

CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	1
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	2
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	4,5
	2b	Specific objectives or hypotheses	6
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	6-11
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	6-11
Participants	4a	Eligibility criteria for participants	6,Table 1, Table 3
	4b	Settings and locations where the data were collected	1
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	8,9
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	6,7,Table 2
	6b	Any changes to trial outcomes after the trial commenced, with reasons	-
Sample size	7a	How sample size was determined	9,10
	7b	When applicable, explanation of any interim analyses and stopping guidelines	9,10
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	8
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	8
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	8
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	8

Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those assessing outcomes) and how	7,8
	11b	If relevant, description of the similarity of interventions	-
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	11-12
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	10
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	-
	13b	For each group, losses and exclusions after randomisation, together with reasons	-
Recruitment	14a	Dates defining the periods of recruitment and follow-up	-
	14b	Why the trial ended or was stopped	-
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	-
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	-
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	-
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	-
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	-
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	-
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	3
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	3
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	-
Other information			
Registration	23	Registration number and name of trial registry	2
Protocol	24	Where the full trial protocol can be accessed, if available	-
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	16

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.

BMJ Open

Study protocol of a Phase 2, Randomized, Placebo-Controlled, Double-Blind, Adaptive, Parallel Group Clinical Study to Evaluate the Efficacy and Safety of Recombinant alpha-1-Microglobulin in Subjects at High Risk for Acute Kidney Injury Following Open-Chest Cardiac Surgery (AKITA Trial)

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Study protocol of a Phase 2, Randomized, Placebo-Controlled, Double-Blind, Adaptive, Parallel Group Clinical Study to Evaluate the Efficacy and Safety of Recombinant alpha-1-Microglobulin in Subjects at High Risk for Acute Kidney Injury Following Open-Chest Cardiac Surgery (AKITA Trial)

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Abstract

Introduction

Acute kidney injury (AKI) is a common complication after cardiac surgery and is associated with adverse short- and long-term outcomes. Alpha-1-microglobulin (A1M) is a circulating glycoprotein with antioxidant-, heme-binding and mitochondrial-protective mechanisms. RMC-035 is a modified, more soluble, variant of A1M and has been proposed as a novel targeted therapeutic protein to prevent cardiac surgery-associated AKI (CS-AKI). RMC-035 was considered safe and generally well tolerated when evaluated in four clinical Phase 1 studies.

Methods and Analysis

This is a Phase 2, randomized, double-blind, adaptive design, parallel group clinical study that evaluates RMC-035 compared to placebo in approximately 268 cardiac-surgical patients at high risk for CS-AKI. RMC-035 is administered as an IV infusion. In total, five doses will be given. Dosing is based on pre-surgery eGFR, and will be either 1.3 or 0.65 mg/kg.

The primary study objective is to evaluate whether RMC-035 reduces the incidence of post-operative AKI, and key secondary objectives are to evaluate whether RMC-035 improves post-operative renal function compared to placebo. A blinded interim analysis with potential sample size re-assessment is planned once 134 randomized subjects have completed dosing. An independent Data Monitoring Committee will evaluate safety and efficacy data at pre-specified intervals throughout the trial. The study is a global multi-center study at approximately 30 sites.

Ethics and dissemination

The trial was approved by the joint ethics committee of the physician chamber Westfalen-Lippe and the University of Münster (code “2021-778-f-A”) and subsequently approved by the responsible ethics committees/relevant institutional review boards for the participating sites. The study is conducted in accordance with Good Clinical Practice, the Declaration of Helsinki, and other applicable regulations. Results of this study will be published in a peer-reviewed scientific journal.

Trial registration number

EudraCT Number: 2021-004040-19

ClinicalTrials.gov Identifier: NCT05126303

Keywords

Acute renal failure, adult intensive & critical care, A1M, cardiac surgery-associated AKI, CS-AKI.

Article Summary

Strengths and limitations of this study

- The trial was designed by a group of global experts in AKI and cardiac surgery, with input from or review by the US Food and Drug Administration and several national medicinal regulatory authorities in Europe and North America.
- This trial uses an adaptive trial design whilst preserving data integrity based on a blinded interim analysis, allowing for re-assessment of statistical assumptions, e.g. AKI event rate, with the possibility to increase the number of patients to be recruited.
- Trial subjects will be recruited at multiple centers in Europe and North America that are considered to reflect standard-of-care in cardiac surgery.
- Limitation: The trial is not powered to show differences in less frequently occurring longer term outcomes such as major adverse kidney events (MAKE).

Introduction

Globally, an estimated 2 million cardiovascular procedures per year are performed.[1] Acute kidney injury (AKI) is considered an important determinant of mortality in patients undergoing cardiovascular surgery.[2] [3] Various epidemiological studies show that cardiac surgery-associated AKI (CS-AKI) occurs in up to 30% of cardiac surgery patients [4] and is associated with an increased morbidity as well as short- and long-term mortality.[5-8] The short-term and long-term mortality rate are 10.7% and 30% respectively and increase along with severity of stages.[6] Heart failure, chronic hyperglycemia, anemia, obesity, preoperative exposure to nephrotoxic drugs or contrast media, inflammation, proteinuria, and pre-existing kidney disease were systematically reviewed and were found to be associated with an increased risk of postoperative CS-AKI.[9]

In clinical studies evaluating potential treatments to prevent CS-AKI in high-risk groups, event rates of AKI up to 65.5% in a high-risk population [10] and 71.7% in patients with a positive biomarker test (that identifies patients at very high risk for AKI) shortly after surgery [11] have been reported. Recent studies on CS-AKI showed that patient enrichment based on the combination of the complexity of surgery and co-morbidities resulted in AKI rates between 28 and 34.2%.[12 13]

Current treatment strategies and clinical management of CS-AKI are mainly supportive, including optimal fluid management and maintenance of hemodynamic stability.[14] At present, no specific therapeutic interventions are available for prevention or treatment of CS-AKI. This is an area of large unmet clinical need, where patients who develop CS-AKI are at risk for additional severe complications, including need for renal replacement therapy (RRT), development of chronic kidney disease (CKD) and mortality. Thus, a single AKI episode after cardiac surgery can lead to a significant burden of comorbidities, prolonged hospital stays, poor quality of life and high long-term costs.

Recombinant alpha-1-microglobulin (RMC-035)

The investigational medicinal product (IMP), RMC-035, contains the pharmacologically active protein RMC-035, which is a recombinant and modified variant of endogenous human alpha-1-microglobulin (A1M). It has four distinct molecular mechanisms, including heme binding, reductase activity, radical scavenging, and mitochondrial binding/protection.[15] Employing several antioxidative mechanisms, it protects cells and tissues from various forms of cell damage caused by oxidative stress, including free radicals and reactive oxygen species (ROS). [16-19]

RMC-035 has been evaluated in four Phase 1 clinical studies: single ascending dose and multiple ascending dose studies in healthy subjects, a renal impairment study, and a phase 1b

pharmacokinetic and safety study in cardiac surgery patients. Available clinical data indicate that RMC-035 is safe and generally well tolerated across study populations and support its continued development in CS-AKI.

The pharmacological characteristics of RMC-035 have are appropriate for the chosen indication of CS-AKI as it targetsmultiple critical disease pathways of CS-AKI, including ischemia-reperfusion injury (IRI), heme toxicity and mitochondrial dysfunction. Furthermore, RMC-035 is rapidly distributed to kidney proximal tubular cells (via glomerular filtration), resulting in high drug exposure in the cells where the primary injury occurs in AKI. It is administered as intravenous (IV) infusion with short systemic half-life which is ideal for hospitalized patients requiring specific renal protection.

Trial objectives

This is a Phase 2, randomized, placebo-controlled, double-blind, adaptive design, parallel group clinical study to evaluate the efficacy and safety of RMC-035 in subjects at high risk for AKI following open-chest cardiac surgery. The study is being conducted to provide proof-of-concept efficacy data and to guide the design of a subsequent pivotal study in this patient population. Based on an integrated assessment of available non-clinical and clinical data, RMC-035 is considered to be well tolerated and is expected to provide targeted renal protection in this patient population, with the aim to prevent or ameliorate peri-operative and post-surgery renal injury.

Primary Objectives are to evaluate RMC-035 for the following outcomes:

• Prevention of AKI (Kidney Disease Improving Global Outcomes [KDIGO] definition [20]) in subjects undergoing cardiac surgery with additional risk factors for developing CS-AKI

• Safety and tolerability

Key secondary objectives are to evaluate RMC-035 for the following outcomes:

• To evaluate RMC-035 for the prevention of post-operative decline (within 72 hours) in renal function

• To evaluate RMC-035 for the reduction of post-operative AKI duration

Other secondary objectives are to evaluate RMC-035 for the following outcomes:

• To evaluate RMC-035 for preserving post-surgery renal function up to Day 90

• To evaluate RMC-035 for the prevention of post-operative dialysis up to Day 90

• To evaluate RMC-035 for the prevention of major adverse kidney events (MAKE) at Days 30 and 90, respectively

• To evaluate RMC-035 for the prevention of AKI within 72 hours (based on cystatin C and/or Urine Output [UO])

• To further evaluate RMC-035 for the prevention, persistence and severity of AKI within 72 hours (based on serum creatinine [SCr]

and/or UO or cystatin C and/or UO)and within 7 days (based on SCr and/or UO or cystatin C and/or UO)

• To evaluate RMC-035 for reducing post-operative albuminuria and proteinuria up to Day 90

• To evaluate the pharmacokinetics of RMC-035

• Identification and characterization of anti-drug-antibodies (ADA) developed after intravenous administration of RMC-035

Exploratory objectives are to evaluate:

• Post-baseline changes in kidney and cardiac biomarkers

• Length of post-operative stay in Intensive Care Unit (ICU) and overall hospitalization time

• Health-related Quality of Life

Methods and analysis

Patients

The target patient population is adults undergoing open-chest cardiac surgery, i.e. CABG and/or valve surgery and/or aorta surgery with additional predisposing risk factors for CS-AKI. Subjects planned to undergo cardiac surgery will be systematically screened for eligibility. Informed consent will be obtained by study site's principal or sub-investigators in all eligible patients before randomization and surgery. Inclusion and exclusion criteria are described in Table 1.

Table 1: Inclusion and Exclusion criteria

Inclusion criteria	A subject is eligible for the study if all of the following apply: 1. Institutional Review Board/ International Ethics Committee approved Informed Consent obtained 2. Ability to understand and comply with the study requirements and able to provide written informed consent 3. Age ≥18 and <85 years

	4. Estimated glomerular filtration rate (eGFR) is ≥30 mL/min/1.73 m2 5. Subject is scheduled for non-emergent coronary artery bypass grafting (CABG) surgery and/or valve surgery and/or ascending aorta aneurysm surgery with use of cardiopulmonary bypass (CPB), and AKI risk factors are present at screening 6. Female subject is not of child-bearing potential, or agreeing not to become pregnant 7. Female subject must not be breastfeeding 8. Female subject must not donate ova 9. Male subject and their female spouse/partner(s) who are of childbearing potential must be using a highly effective form of birth control 10. Male subjects must not donate sperm 11. Subject agrees not to participate in another interventional study
Exclusion criteria	Subject will be excluded from participation if any of the following apply: 1. Medical condition that makes the subject unsuitable for study participation 2. Scheduled for emergent surgeries (eg, aortic dissection) 3. Scheduled for CABG and/or valve surgery and/or ascending aorta aneurysm surgery combined with additional non-emergent cardiac surgeries (eg, congenital heart defects) 4. Scheduled to undergo transcatheter aortic valve implantation (TAVI) or transcatheter aortic valve replacement (TAVR), or off-pump surgeries or left ventricular assist device (LVAD) implantation 5. Experiences a cardiogenic shock or hemodynamic instability which require inotropes or vasopressors or other mechanical devices within 24 hours prior to surgery 6. Requirement for defibrillator or permanent pacemaker, mechanical ventilation, intraaortic balloon pumping (IABP), LVAD, or other forms of mechanical circulatory support (MCS) 7. Diagnosed with AKI (as defined by KDIGO criteria) within 3 months prior to surgery

	8. Required cardiopulmonary resuscitation within 14 days prior to cardiac surgery 9. Ongoing sepsis or an untreated diagnosed clinically significant infection (viral or bacterial) 10. Total bilirubin or alanine aminotransferase (ALT) or aspartate aminotransferase (AST) ≥ 2 times the upper limit of normal (ULN) 11. History of solid organ transplantation 12. History of renal replacement therapy (RRT) 13. Medical condition which requires active immunosuppressive treatment 14. Ongoing chemotherapy or radiation therapy for malignancy that may have an impact on kidney function 15. Received an investigational medicinal product within the last 90 days (or within 5 half-lives of the investigational drug, whichever is longer) 16. Subject has a known allergy to RMC-035 or one of its constituents, or has previously received RMC-035

Main outcomes

Primary Endpoints

Primary Efficacy Endpoint

Proportion of patients developing AKI within 72 hours after first dose of IMP, based on SCr and/or UO (AKI of any stage/severity according to KDIGO definition, ie, SCr ≥1.5 times baseline, or increase of SCr of ≥0.3 mg/dL [≥26.5 µmol/L], or UO <0.5 mL/kg/h for ≥6 hours).

Primary Safety Endpoint

Nature, frequency and severity of treatment-emergent adverse events (TEAEs; defined as any AE which occur within 72 hours after last IMP administration).

Secondary Endpoints

Key Secondary Endpoints

Time-corrected area under the curve (AUC) of SCr for Day 1 to Day 4 (72 hours after first dose of IMP)

Duration of AKI defined as the number of days meeting the definition of AKI (KDIGO definition) starting within 72 hours after first dose of IMP until resolution.

For other Secondary Endpoints and Exploratory Endpoints, please see Table 2.

Table 2: Other Secondary Endpoints and Exploratory Endpoints

Other Secondary Endpoints	- Post-baseline changes in renal function
	  - SCr and cystatin C (and corresponding eGFR values) at 12, 24,48, and 72 hours, respectively, and at Day 7/discharge, Day 30 and Day 90
	  - Change from baseline, up to Day 7/discharge, of peak SCr and cystatin C
	- Time-corrected AUC of cystatin C for Day 1 to Day 4 (72 hours after first dose of IMP)
	- Need for renal replacement therapy
	  - Dialysis treatment (for any reason) within 72 hours and within 7 days after end of surgery
	  - Dialysis free days from end of surgery to Day 30 and Day 90, respectively
	- MAKE at Day 30 and Day 90, defined as death, any dialysis, or ≥25% reduction of eGFR compared to baseline Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation (either SCr, cystatin C, or both)[21-23]
	- AKI Characteristics
	  - AKI within 72 hours after first dose of IMP based on cystatin C and/or UO (AKI of any stage/severity defined as cystatin C ≥1.5 baseline, OR UO <0.5 mL/kg/h for ≥6 hours)
	  - AKI within 7 days after first dose of IMP (based on SCr and/or UO criteria, or cystatin C and/or UO criteria)
	  - AKI persistence, defined as an AKI (KDIGO definition) developing within 72 hours after first dose of IMP and with a duration of ≥72 hours. Persistence will also be assessed per AKI severity stage*
	  - AKI severity stage* within 72 hours and within 7 days after first dose of IMP
	  - *Severity of AKI defined as the following:
	  -Stage 1: SCr 1.5 to 1.9 times baseline within 7 days, OR ≥0.3 mg/dL (≥26.5 µmol/L), OR urine output <0.5 mL/kg/h

	for 6 to <12 hours -Stage 2: SCr 2.0-2.9 times baseline within 7 days OR urine output <0.5 mL/kg/h for ≥12 hours -Stage 3: SCr 3.0 times baseline within 7 days, OR increase in SCr 4.0 mg/dL (≥353.6 µmol/L), OR initiation of renal replacement therapy OR urine output <0.3 mL/kg/h for ≥24 hours OR anuria for ≥12 hours
Exploratory Endpoints	- Post-baseline changes in urine albumin to creatinine ratio (UACR) and urine protein to creatinine ratio (UPCR) at Day 4, Day 30, and Day 90 - Pharmacokinetics of RMC-035 in plasma (AUC and Cmax) - Presence and titers of ADA at Day 1 (pre-surgery), Day 30, and Day 90 - Characteristics of ADA developed at Day 30 and Day 90 with regards to isotype, neutralizing capacity, and cross-reactivity with endogenous alpha-1-microglobulin (A1M) **Post-baseline changes in kidney and cardiac biomarkers** - Kidney biomarkers: Urine kidney injury molecule 1 (KIM-1), neutrophil gelatinase-associated lipocalin (NGAL), tissue inhibitor of metalloproteinase 2 (TIMP2), insulin like growth binding factor protein 7 (IGFBP7), chemokine ligand 14 (CCL-14), interleukin-18 (IL-18), liver fatty acid binding protein (LFABP) and 8-hydroxy-2'-deoxyguanosine (8-OHdG) - Cardiac Biomarkers: Plasma N-terminal-pro-hormone BNP (NT-pro BNP) and cardiac troponin I and T (cTnI, cTnT) **Hospitalization time and discharge facility** - Length of index ICU stay and index hospital stay - Index ICU stay (in Days) defined as the duration of stay in the ICU Immediately following surgery or recovery room post-surgery until ICU discharge - Index hospital stay (in Days) is defined as the duration of stay in the hospital from the day of surgery to hospital discharge for the index surgery - Nature of subject discharge facility (eg, home, skilled nursing facility, Rehabilitation center) **Health-related Quality of Life assessments** - Change from baseline to Day 90 in the following Patient Reported

	Outcomes (PROs): - MOS 36-Item Short Form Survey Instrument (SF-36) - European Quality of Life 5 Domain 5-Level Score (EQ-5D-5L)

Trial oversight

A scientific advisory board of experts in anesthesiology and cardiac surgery has been established by the sponsor to facilitate design of the trial, provide medical scientific leadership and make trial-specific recommendations to the sponsor. Likewise, an independent unblinded data monitoring committee (DMC) consisting of experts in acute kidney injury and a statistician will perform review of safety findings at regular intervals during the study. The working procedures of the DMC are defined in a DMC charter; their safety evaluation will be based on all available accumulated safety data. The DMC will also be responsible for recommendation on the study conduct following the interim analysis (IA) based upon predefined unblinded statistical output. Communication and recommendations by the DMC will be blinded. Completeness and accuracy of the data will be monitored by a Clinical Research Organization which performs regular monitoring visits.

Patient and public involvement statement

Reflecting the acute and unpredictable nature of CS-AKI there are no specific patient advocacy groups at present; closest are organizations that represent kidney diseases after cardiac surgery in general and, although they are aware of our program, they were not specifically consulted on the design of this study.

Trial design

Overall study design

This is a Phase 2, multi-center, randomized, double-blind, placebo-controlled, adaptive design, parallel group trial including patients undergoing open-chest cardiac surgery. The study consists of a screening period of up to 30 days before surgery, five in-hospital visits during the anticipated post-operative hospitalization period, and two additional follow-up visits at Day 30 and Day 90, respectively. A study flow chart is shown in Figure 1 and the schedule of study specific assessments is presented in Table 3. The study reporting was assessed following SPIRIT guidelines and is presented in the SPIRIT checklist in the supplementary material.

Table 3: Schedule of Assessments – Complete Study Protocol

Assessments	**Screening**	**Treatment**							**Follow-Up**			
Visit Number	**1** [h]	**2 Day of Surgery**					**3**	**4 (EOT)**	**5** [u]	**6 (discharge)** [v]	**7** [w]	**8 (EoS)** [w, x]
Visit Day	**-30 to -1**	**1**					**2 (24h)**	**3 (48h)**	**4 (72h)**	**7**	**30**	**90**
Allowed visit window (days)	**±0**	**±0**					**±0**	**±0**	**±0** [q]	**±2**	**±3**	**±7**
Visit hour (h)		pre	0h	1h	2h	6h [y]	12 h [y]					
Informed consent	x											
Inclusion/exclusion criteria	x	x										
Medical history	x											
Demographics	x											
Weight and height [a]	x						x	x	x			
Physical examination [b]	x								x			
Pregnancy test (WOCBP only) [c]	x	x										
Hematology lab [d]	x						x	x	x			
Clinical chemistry lab [d]	x						x	x	x			
Liver function lab [d]	x						x	x	x			
Serum creatinine (SCr) [e]	x	x					x	x	x	x	x	x
Serum Cystatin C [f]	x	x					x	x	x	x	x	x
UACR and UPCR [g]	x	x							x		x	x
Urinalysis		x							x			
Randomization [h]	x											
Record renal replacement therapy							x	x	x		x	x
IMP administration [i]			x [j]			x [k]	x [l]	x [l]	x [l]			
Plasma PK sampling		x		x	x			x, x, x [m]	x, x, x [m]			
12-lead electrocardiogram	x	x						x	x	x		
Vital signs [n]	x	x						x	x	x		
Surgery assessments [o]			←			→						
Discharge from ICU [p]							←					→

Urine output [q]		←										→
Urine sampling for biomarkers [r]		x				x		x	x				
Plasma/serum sampling for biomarkers [r]		x						x		x			
HRQoL Assessments [s]	x												x
ADA assessment		x										x	x
Concomitant medication recording [t]	←												→
AE recording				←									→
SAE recording		←											→

Abbreviations: ADA = anti-drug antibody; AE = adverse event; AKI = acute kidney injury; EOS = End of Study; EOT = End-of-Treatment; EQ-5D-5L = European Quality of Life 5 Domain 5-Level Score; HRQoL = health related quality of life; ICU = intensive care unit; IMP = investigational medicinal product; PK = pharmacokinetic; PRO = patient reported outcome; SAE = serious adverse event; SCr = serum creatinine; WOCBP = woman of childbearing potential; UACR = urine albumin to creatinine ratio; UAPR = urine albumin to protein ratio

a. Height only measured at screening (Visit 1). Weight during ICU stay only required if possible.

b. The initial physical examination performed at screening should be comprehensive; all other physical examinations may be abbreviated and symptom driven.

c. A serum pregnancy test completed during the screening period within 48 hours prior to surgery does not need to be repeated on the day of surgery. If the serum pregnancy test occurs more than 48 hours prior to the date of surgery, a serum or urine pregnancy test will also be performed on Day 1 prior to surgery.

d. Hematology Labs: Hematocrit, Hemoglobin (Hb), Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration, Red cell distribution width, Red blood cells, Platelets, Leucocytes (including Neutrophils, Monocytes, Lymphocytes, Eosinophils, Basophils)
Clinical Chemistry Labs: Albumin, Calcium, Chloride, Serum creatinine (SCr), C-reactive protein (CRP), Sodium, estimated glomerular filtration rate (eGFR), Magnesium, Phosphate, Potassium, Blood urea nitrogen (BUN), Uric acid, Glucose
Liver Function Labs: Alanine aminotransferase (ALAT), Alkaline phosphatase (ALP), Aspartate aminotransferase (AST), Bilirubin (total and conjugated), Gamma glutamyltransferase (GGT)

e. The screening sample for SCr must be collected on Day -1 (or day of surgery, see **footnote h**) and will be analyzed locally (to evaluate eligibility and determine correct start dose of RMC-035 according to renal function) and centrally (as baseline for endpoint assessment). All SCr samples collected during hospital stay will be analyzed both locally (to support AKI evaluation) and in a central lab (for the purpose of endpoint assessments). Day 30 and 90 samples will be analyzed centrally.

f. Cystatin C samples will be collected and analyzed in a central lab only for the purpose of endpoint assessments

g. UACR: screening sample will be collected as a spot urine sample and analyzed locally to evaluate albuminuria as an eligibility criterion (in the absence of historical albuminuria data within 3 months prior to randomization). UACR and UPCR: In-hospital samples (Day 1, Visit 2 and Day 4, Visit 5) will be collected either as a First Morning Void (FMV) sample or drawn directly from a Foley catheter and analyzed in a central lab only. Follow-up samples (Day 30, Visit 7 and Day 90, Visit 8) will be collected as FMV samples as possible and analyzed in a central lab only.

h. Randomization must occur on Day -1, ie the day before surgery is intended. All screening assessments may be performed on Day 1 prior to surgery, including randomization. These assessments must be completed prior to any pre-surgical activities, such as administration of fluids or medications, including anesthesia.

i. All 5 doses to be calculated using the same weight measurement that is used for randomization / stratification. IMP will be permanently discontinued in subjects developing AKI stage 2 or higher as per KDIGO guidelines

j. IV infusion over 60 minutes, first infusion should start approximately 10 minutes before expected onset of CPB (time point 0 is defined as start of IMP administration)

k. IV infusion over 60 minutes at 6 h (±30 min) after the start of first infusion

l. IV infusion over 30 minutes at 12 h, 24 h and 48 h (±30 min) after the start of first infusion

m. PK sampling at Day 2 and 3 should occur 30 min (±5 min) and 90 min (±15 min) from start of IMP infusion

Plasma PK Sampling Study Day	Time Window	**Predose** ≤30 min	**30 min** ±5 min	**1 h** ±5 min	**90 min** ±15 min	**2 h** ±15 min
Day 1	Start of Infusion 1 (t=0 h)	x		x		x
Day 2	Start of Infusion 4 (t=24 h)	x	x		x	
Day 3	Start of Infusion 5 (t=48 h)	x	x		x	

n. Vital signs: body temperature, blood pressure, heart rate, respiratory rate, SpO2

o. Data points to collect are type of CPB pump (pulsatile or non-pulsatile, if applicable) and duration of CPB (exact time of initiation and end of CPB), duration of surgery (beginning of surgery defined as exact time of initial skin incision, end of surgery defined as exact time of skin closure), blood loss volume, administration of any fluids during surgery (blood products [red blood cells, plasma, cryoprecipitate, platelets, etc.], crystalloids, colloids, and others), target body temperature during CPB and time at temperature range, duration of cross clamp (minutes), number, position, and graft source bypasses performed, length of time with mean arterial pressure <50 mmHg, valve surgery type (replacement or repair), replacement valve origin (bioprosthetic or mechanical), aortic repair type, and time of admission to the ICU.

p. Time of discharge from ICU to hospital ward, another treatment facility or home

q. Only required as long as Foley catheter is in place

r. Urine samples for evaluation of exploratory urinary (kidney) biomarkers and plasma/serum samples for evaluation of cardiac biomarkers will be collected at the intervals described below, respectively:

Biomarker Sampling Time Window (in relation to Start of Infusion 1 (t=0 h))	**Predose** ≤60 min	**6 h** ±30 min	**24 h** ±30 min	**48 h** ±30 min	**72 h** ±30 min
Urine	x	x	x	x	
Plasma/serum	x		x		x

s. PRO HRQoL assessment: SF 36 and EQ-5D-5L. PRO HRQoL assessments to be performed as early as possible in the screening period.

t. Medications taken within 30 days prior to the day surgery is intended are to be collected. Use of contrast agent within 72 hours prior to the day surgery is intended should be documented as a prior/concomitant medication. When possible, type and quantity of contrast agent should be recorded.

u. Visit 5 must occur at 72 hours from start of first infusion of IMP, with a scheduling window of +/-2 hours.

v. Visit 6 and all associated assessments should occur on the day of hospital discharge. In case subject is discharged on Day 4, discharge (Visit 6) assessments performed prior to discharge on that day are acceptable.

w. Visit may be performed by qualified and trained study staff at the subject's home or other suitable location, where appropriate

x. In case of subject withdrawal, subject should be encouraged to undergo all EOS assessments as an Early Termination visit.

y. Assessments must be performed prior to IMP administration

Randomization procedure

The trial population consists of patients at high risk for AKI and undergo cardiac surgery with additional predisposing risk factors for CS-AKI (inclusion and exclusion criteria are shown in Table 1). Eligible subjects will be randomized to receive either RMC-035 or placebo in a 1:1 randomization ratio. Both region (EU versus North America) and pre-operative eGFR calculated using the CKD-EPI equation with SCr on local laboratory results (≥60 and <60 mL/min/1.73m^2) will be used as stratification factors to ensure a balanced randomization within these groups. Randomization will be performed by the investigators using the centralized electronic randomization platform. Randomization codes will be subsequently computer-generated and sent to study pharmacists for IMP preparation.

IMP presentation and blinding

The Investigator, study personnel, and subject will be blinded to the identity of the IMP (RMC-035 or placebo). The investigational pharmacist will be responsible for the preparation of IMP for each subject and will be unblinded to the randomization assignment. In the event of a medical emergency requiring knowledge of the treatment assigned to the subject, unblinding can be performed and the treatment code for a given randomized subject will be provided. The time, date, subject number and reason for obtaining any of these codes, and therefore breaking the blind, will be documented in the study file. The treatment code can only be requested by the Investigator or other persons designated as sub-investigators. No subjects or study personnel will be made aware of the treatment given unless a medical emergency necessitates such disclosure. Both IMP and placebo will be presented in 50ml syringes and labelled in a standardized manner. Label information includes study protocol number, subject-ID, syringe number, date and time of IMP preparation, IMP content (RMC-035 or placebo) and sponsor contact information, but no information about randomization group. Both RMC-035 and placebo are transparent, odorless fluids that cannot be differentiated optically or by other sensations. Pharmacokinetic samples taken at the study sites and evaluated in a central laboratory of the sponsor will be used to determine systemic exposure to study drug.

IMP dosing

In total five IV doses of either RMC-035 or placebo will be administered during the study. The first dose will start approximately 10 minutes before cardiopulmonary surgery is initiated (0h), and subsequent doses will be given at 6, 12, 24, and 48 hours later. IMP will be given as a continuous IV infusion over either 60 minutes (first two doses) or 30 minutes for subsequent doses. Due to the pharmacokinetic properties of RMC-035, the predefined dose levels (prior to surgery) are based on renal function (eGFR by CKD-EPI equation with SCr) at screening (Day -1, Visit 1): 1) subjects with eGFR ≥60 mL/min/1.73m^2 will receive 1.3 mg/kg (per dose) for the first and second dose, followed by 0.65 mg/kg (per dose) for the third, fourth and fifth

dose; 2) Subjects with eGFR >30 and <60 mL/min/1.73m^2 will receive 0.65 mg/kg (per dose) for all five doses. The trial drug is provided on top of standard of care of each center.

Permanent discontinuation of IMP

Local laboratory results will be utilized for clinical care and real time evaluation of AKI for purposes of IMP discontinuation. Subjects who meet any of the following IMP discontinuation criteria during the treatment period will be discontinued from IMP and continue study participation and procedures and follow up as per the schedule of assessment until the end of the study visit: 1) Development of stage ≥2 AKI, according to KDIGO definition, 2) Need for RRT, 3) Need for percutaneous or surgical mechanical circulatory support (MCS) or extra-corporeal membrane oxygenator (ECMO), 4) Reporting of a grade 3 (per Common Terminology Criteria for Adverse Events [CTCAE]) or higher adverse event of ISR or IRR, and which is considered to be an immune-mediated reaction

The IMP will be discontinued in case of any of the following abnormal liver chemistry tests in blood: 1) ALT > 3 × ULN combined with total bilirubin > 2 × ULN in the same sample, 2) ALT > 3 × ULN if associated with symptoms (new or worsening) believed to be related to hepatitis (such as fatigue, nausea, vomiting, right upper quadrant pain or tenderness or jaundice) or believed to be related to hypersensitivity (such as fever, rash or eosinophilia), 3) ALT > 3 x ULN if confirmed in a second sample within 24 hours AND in the second sample International Normalized Ratio (INR) is increased to >1.5 x ULN, 4) ALT > 8 x ULN in any individual sample during the treatment period All liver chemistry abnormalities as summarized above need to be followed up regularly (at least every 24 hours) until values have returned to baseline or are considered stable. If IMP is discontinued, the subject will remain in the study to be evaluated until the end of the study Visit (as much as possible) or complete the end of the study visit assessments.

Planned sample size & interim analysis (IA)

A minimum of 268 and a maximum of 348 patients are planned to be randomized at approximately 30 sites predominantly across Europe and North America. The final number of patients randomized will depend on the results of the IA.

There will be one IA during the trial which will be conducted once 50% of the planned randomized subjects have completed visit 6 (Day 5-9). Sample size may be increased to a maximum of 348 randomized subject but will not be decreased. The study may be stopped at the IA for futility or safety reasons. Study eligibility criteria may also be modified.

Safety reporting

Safety is followed up until last study visit at Day 90. Serious adverse events (SAEs) will be reported to the sponsor and other stakeholders as per regulatory requirements (eg, competent authorities and/or IRBs/CECs, when applicable). Non-serious AEs will only be reported up to Day 30 Treatment emergent AEs (TEAEs) are defined as AEs which occur after the initial IMP administration through 72 hours after last IMP administration. Safety reporting will be managed by a clinical research organization in compliance with regulatory requirements of each region of the study.

Statistical Considerations

For the primary endpoint, KDIGO AKI (serum creatinine and urine output) within 72 hours after first dose of IMP, RMC-035 has been assumed to lead to a 30% relative risk reduction vs. placebo. The event rate in the placebo group has been assumed to be 50%.[24] A sample size of 268 subjects randomized leads to a test power of 80% to show statistically significant results at a two-sided significance level (alpha) of 0.10.

The primary endpoint will be analyzed by the Cochran-Mantel-Haenszel estimate of the common relative risk (RMC-035 vs. placebo) across the four stratification groups formed by region and pre-operative eGFR (≥60 and <60 mL/min/1.73m2). In addition, the proportion of subjects with AKI within 72 hours after first dose of IMP and its 90% confidence interval will be calculated for each treatment group. The hypotheses to be tested are as follows: 1) Null hypothesis: the proportion of subjects developing AKI within 72 hours after first dose of IMP is the same for the RMC-035 and the placebo treatment groups ($\mathbf{p}_{\text{RMC-035}} = \mathbf{p}_{\text{placebo}}$), 2) Alternative hypothesis: the proportion of subjects developing AKI within 72 hours after first dose of IMP is different for the RMC-035 and the placebo treatment groups ($\mathbf{p}_{\text{RMC-035}} \neq \mathbf{p}_{\text{placebo}}$).

All secondary endpoints have a supplementary character and will be reported with no strict control of the type I error. Secondary continuous efficacy endpoints that are measures of renal function will be analyzed using robust regression of log-transformed values having sequentially imputed any missing data using multiple imputation. A sensitivity analysis will be performed using a mixed model for repeated measurements (MMRM) if data are approximately normally distributed.

For the key secondary AUC endpoint, the geometric least square means of the time-corrected AUC of SCr for Days 1 to 4 will be obtained for each, RMC-035 and placebo, by transforming the model estimates back to the original scale.

Supportive analyses of renal function will be performed on cystatin C levels (and corresponding eGFR values assessed by Chronic Kidney Disease Epidemiology Collaboration [CKD-EPI] equations with either SCr, cystatin C, or both) and cumulative AUC values up to each timepoint. Secondary binary efficacy endpoints will be analyzed using the same approach as specified above for the primary endpoint.

Further details on the IA and the control of the type I error will be provided in the DMC Charter and the Statistical Analysis Plan (SAP).

Safety Endpoints

Safety analyses will be conducted on the Safety Analysis Set (SAF), which includes all randomized subjects who received at least 1 dose of IMP. To characterize the safety profile, the number and percentage of subjects with TEAEs and AEs per dose group will be tabulated. Descriptive statistics will be provided for laboratory tests (hematology, biochemistry, urinalysis) and vital signs (pulse rate, respiratory rate and blood pressure) by visit and for the changes from baseline to each visit split by treatment group. In addition, the development of anti-drug antibodies after intravenous administration of RMC-035 will be investigated and characterized.

Pharmacokinetics

Descriptive statistics will be presented for plasma concentrations by scheduled sample time. Specific pharmacokinetic parameters will be presented separately. Summaries will be provided by dose group, age group and by renal function prior to surgery.

Sensitivity analyses and secondary endpoints

Secondary continuous efficacy endpoints that are measures of renal function will be analyzed using robust regression of log-transformed values having sequentially imputed any missing data using multiple imputation. A sensitivity analysis will be performed using a mixed model for repeated measurements (MMRM) if data are approximately normally distributed.

For the key secondary AUC endpoint, the geometric least square means of the time-corrected AUC of SCr for Days 1 to 4 will be obtained for each, RMC-035 and placebo, by transforming the model estimates back to the original scale. The relative difference (RMC-035 vs. placebo) and its 90% confidence interval will also be reported.

Supportive analyses of renal function will be performed by using cystatin C levels (and corresponding eGFR values assessed by Chronic Kidney Disease Epidemiology Collaboration [CKD-EPI] equations with either SCr, cystatin C, or both) and cumulative AUC values up to each timepoint.

Secondary binary efficacy endpoints will be analyzed using the same approach as specified above for the primary endpoint.

Additional sensitivity analyses of the primary and secondary endpoints, and evaluation of exploratory endpoints, will be detailed in the SAP.

Ethics and dissemination

This trial will be conducted in accordance with the protocol and consensus ethical principles derived from international guidelines including the Declaration of Helsinki and Council for International Organizations of Medical Sciences (CIOMS) International Ethical Guidelines; applicable International Conference on Harmonization (ICH) GCP Guidelines; and applicable laws and regulations. The protocol, substantial protocol amendments, ICF, IB, and other relevant documents (e.g., any other written information regarding this trial to be provided to the patient or the patient's legal representative) are approved by the Ethics Committee of the Physicians Chamber Westfalen-Lippe and the University of Münster in Germany (file number 2021-778-f-A).

Informed consent will be given by patients before any study relevant procedure is performed. Patients will be assigned a unique patient identification number. Any patient records or datasets that are transferred to the Sponsor will contain this identifier only; patient names and any information which would make the patient identifiable will not be transferred. All laboratory specimens, evaluation forms, reports, and other records will be identified in a manner designed to maintain patient confidentiality.

Irrespective of whether the trial is completed or prematurely terminated, the sponsor will ensure that the trial results will be posted on publicly available clinical trial registries in accordance with their requirements. In addition, results will be presented at international congresses and published in peer-reviewed journals. Participant-level access to data will not be granted to the public.

Conclusions

The ongoing Phase 2 (AKITA) study is specifically designed to answer the primary research question whether the investigational drug RMC-035 can reduce the occurrence of CS-AKI within 3 days after open cardiac surgery. Study results will also provide pertinent information on other clinically relevant endpoints, such as the severity, duration and persistence of AKI, changes in post-operative renal function and major adverse kidney events (MAKE), and will significantly expand the current knowledge of the safety profile of RMC-035. Finally, biomarker analysis may improve the understanding of its mechanism of action, shed light on relevant disease pathways in CS-AKI and facilitate the design of future clinical trials of RMC-035.

Author's contributions

AZ, CDM, TEL, and RR co-drafted the manuscript. NSF, JB, FW, AM, CCDB, JLK, AB, DTE, BdV, NN, MT, AL, ML, TvG and CR participated in trial protocol design, reviewed and edited the manuscript.

Funding statement

This work was supported by Guard Therapeutics.

Competing interest statement

Alexander Zarbock received advisory board consultancy and travel reimbursements from Guard Therapeutics.

Data availability statement: Data obtained during the trial is the property of the study sponsor and will be handled in compliance with data protection policies and regulations.

Word count:

3273 words

Figure legend

Figure 1: study flowchart

Ethics Approval

The study was approved by the Ethics Committee of the Physicians Chamber Westfalen-Lippe and the University of Münster in Germany (file number 2021-778-f-A).

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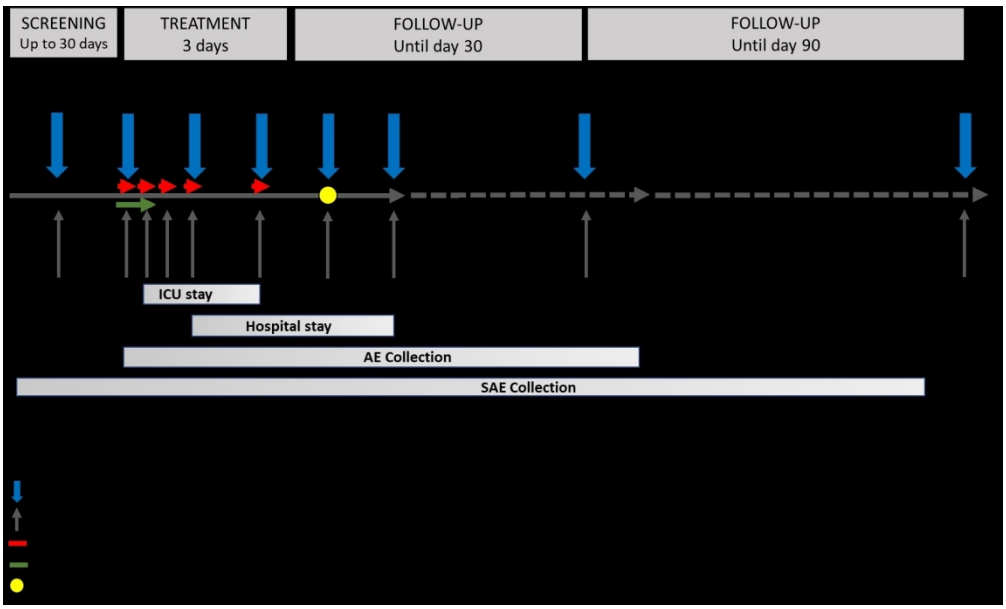

Figure 1: Study flowchart

307x183mm (150 x 150 DPI)

Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

In your methods section, say that you used the SPIRITreporting guidelines, and cite them as:

Chan A-W, Tetzlaff JM, Gøtzsche PC, Altman DG, Mann H, Berlin J, Dickersin K, Hróbjartsson A, Schulz KF, Parulekar WR, Krleža-Jerić K, Laupacis A, Moher D. SPIRIT 2013 Explanation and Elaboration: Guidance for protocols of clinical trials. BMJ. 2013;346:e7586

	Reporting Item		Page Number
Administrative information			
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
11Trial registration	#2a	Trial identifier and registry name. If not yet registered, name of intended registry	2
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	2
Protocol version	#3	Date and version identifier	n/a
Funding	#4	Sources and types of financial, material, and other support	12
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	1
Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	1
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication,	12

		including whether they will have ultimate authority over any of these activities	
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	7
Introduction			
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	4,5
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	n/a
Objectives	#7	Specific objectives or hypotheses	5,6,7
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	8-11
Methods: Participants, interventions, and outcomes			
Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	9
Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	6, Table 1
Interventions:	#11a	Interventions for each group with sufficient detail to	7,8

description		allow replication, including how and when they will be administered	
Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	8,9
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	8,11
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	Table 1
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	5-7
Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	Table 3
Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	9
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	9
Methods: Assignment of interventions (for controlled trials)			
Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of	8

		any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	
Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	8
8Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	8
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	8
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	8
Methods: Data collection, management, and analysis			
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	10,11
Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	7

Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	7
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	10-12
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	11,12
Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	11,12
Methods: Monitoring			
Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	7
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	10
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	10
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent	10

		from investigators and the sponsor	
Ethics and dissemination			
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	2,12
Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	n/a
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	6,12
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	n/a
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	7,12
Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	13
Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	13
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	n/a
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	12

Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	n/a
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	12
Appendices			
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	Supplementary material
Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	n/a

CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	1
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	2
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	4,5
	2b	Specific objectives or hypotheses	6
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	6-11
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	6-11
Participants	4a	Eligibility criteria for participants	6,Table 1, Table 3
	4b	Settings and locations where the data were collected	1
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	8,9
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	6,7,Table 2
	6b	Any changes to trial outcomes after the trial commenced, with reasons	-
Sample size	7a	How sample size was determined	9,10
	7b	When applicable, explanation of any interim analyses and stopping guidelines	9,10
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	8
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	8
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	8
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	8

Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those assessing outcomes) and how	7,8
	11b	If relevant, description of the similarity of interventions	-
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	11-12
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	10
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	-
	13b	For each group, losses and exclusions after randomisation, together with reasons	-
Recruitment	14a	Dates defining the periods of recruitment and follow-up	-
	14b	Why the trial ended or was stopped	-
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	-
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	-
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	-
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	-
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	-
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	-
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	3
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	3
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	-
Other information			
Registration	23	Registration number and name of trial registry	2
Protocol	24	Where the full trial protocol can be accessed, if available	-
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	16

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.

SPIRIT
STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Page no.
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	2
	2b	All items from the World Health Organization Trial Registration Data Set	2
Protocol version	3	Date and version identifier	n/a
Funding	4	Sources and types of financial, material, and other support	20
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1
	5b	Name and contact information for the trial sponsor	20
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	20
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	11
Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	4-5
	6b	Explanation for choice of comparators	5
Objectives	7	Specific objectives or hypotheses	5-6

Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	11-12
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	1
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	6-8
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	11-16
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	16-17
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	18
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	7-8
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	8-11
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	11-15
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	17-18
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	n/a
Methods: Assignment of interventions (for controlled trials)			
Allocation:			

Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	15
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	15
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	15
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	15-16
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	15-16
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	17-18
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	n/a
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	19
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	17-19
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	18-19
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	n/a

Methods: Monitoring

Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	11-17
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	11
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	17
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	17

Ethics and dissemination

Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	19
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	19
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	19
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	n/a
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Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	n/a

Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	19
	31b	Authorship eligibility guidelines and any intended use of professional writers	n/a
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BMJ Open

Study protocol of a Phase 2, Randomized, Placebo-Controlled, Double-Blind, Adaptive, Parallel Group Clinical Study to Evaluate the Efficacy and Safety of Recombinant alpha-1-Microglobulin in Subjects at High Risk for Acute Kidney Injury Following Open-Chest Cardiac Surgery (AKITA Trial)

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	Anesthesiology, Intensive Care Medicine Ronco, Claudio; San Bortolo Hospital of Vicenza, International Renal Research Institute of Vicenza Zarbock, Alexander; University Hospital Münster Department of Anesthesiology and Intensive Care Medicine
Primary Subject Heading:	Anaesthesia
Secondary Subject Heading:	Cardiovascular medicine, Anaesthesia, Intensive care, Renal medicine, Surgery
Keywords:	Cardiac surgery < SURGERY, Acute renal failure < NEPHROLOGY, Adult intensive & critical care < INTENSIVE & CRITICAL CARE

Study protocol of a Phase 2, Randomized, Placebo-Controlled, Double-Blind, Adaptive, Parallel Group Clinical Study to Evaluate the Efficacy and Safety of Recombinant alpha-1-Microglobulin in Subjects at High Risk for Acute Kidney Injury Following Open-Chest Cardiac Surgery (AKITA Trial)

C. David Mazer [1,2,3], Niloufar Siadati-Fini,[1] Johannes Boehm [4,5], Felix Wirth [4,5], Andrej Myjavec [6], C Craig D. Brown [7], Jay L. Koyner [8], Andreas Boening [9], Daniel T. Engelman [10], Tobias E. Larsson [11], Ronny Renfurm [12], Benoit de Varennes [13], Nicolas Noiseux [14], Matthias Thielmann [15], Andre Lamy [16,17], Maxime Laflamme [18], Thilo von Groote [19], Claudio Ronco [20], Alexander Zarbock [19]

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5 Insure (Institute for Translational Cardiac Surgery), Department of Cardiovascular Surgery, German Heart Center Munich, Munich, Germany.
6 Department of Cardiac Surgery, University of Hradec Kralove, Hradec Kralove, Czech Republic
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16 Department of Surgery, McMaster University, Hamilton, Ontario, Canada.
17 Population Health Research Institute, Hamilton Health Sciences, Hamilton, Ontario, Canada.
18 Institut universitaire de cardiologie et de pneumologie, Quebec, Canada.
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Abstract

Introduction

Acute kidney injury (AKI) is a common complication after cardiac surgery and is associated with adverse short- and long-term outcomes. Alpha-1-microglobulin (A1M) is a circulating glycoprotein with antioxidant-, heme-binding and mitochondrial-protective mechanisms. RMC-035 is a modified, more soluble, variant of A1M and has been proposed as a novel targeted therapeutic protein to prevent cardiac surgery-associated AKI (CS-AKI). RMC-035 was considered safe and generally well tolerated when evaluated in four clinical Phase 1 studies.

Methods and Analysis

This is a Phase 2, randomized, double-blind, adaptive design, parallel group clinical study that evaluates RMC-035 compared to placebo in approximately 268 cardiac-surgical patients at high risk for CS-AKI. RMC-035 is administered as an IV infusion. In total, five doses will be given. Dosing is based on pre-surgery eGFR, and will be either 1.3 or 0.65 mg/kg.

The primary study objective is to evaluate whether RMC-035 reduces the incidence of post-operative AKI, and key secondary objectives are to evaluate whether RMC-035 improves post-operative renal function compared to placebo. A blinded interim analysis with potential sample size re-assessment is planned once 134 randomized subjects have completed dosing. An independent Data Monitoring Committee will evaluate safety and efficacy data at pre-specified intervals throughout the trial. The study is a global multi-center study at approximately 30 sites.

Ethics and dissemination

The trial was approved by the joint ethics committee of the physician chamber Westfalen-Lippe and the University of Münster (code “2021-778-f-A”) and subsequently approved by the responsible ethics committees/relevant institutional review boards for the participating sites. The study is conducted in accordance with Good Clinical Practice, the Declaration of Helsinki, and other applicable regulations. Results of this study will be published in a peer-reviewed scientific journal.

Trial registration number

EudraCT Number: 2021-004040-19

ClinicalTrials.gov Identifier: NCT05126303

Keywords

Acute renal failure, adult intensive & critical care, A1M, cardiac surgery-associated AKI, CS-AKI.

Article Summary

Strengths and limitations of this study

- The trial was designed by a group of global experts in AKI and cardiac surgery, with input from or review by the US Food and Drug Administration and several national medicinal regulatory authorities in Europe and North America.
- This trial uses an adaptive trial design whilst preserving data integrity based on a blinded interim analysis, allowing for re-assessment of statistical assumptions, e.g. AKI event rate, with the possibility to increase the number of patients to be recruited.
- Trial subjects will be recruited at multiple centers in Europe and North America that are considered to reflect standard-of-care in cardiac surgery.
- Limitation: The trial is not powered to show differences in less frequently occurring longer term outcomes such as major adverse kidney events (MAKE).

Introduction

Globally, an estimated 2 million cardiovascular procedures per year are performed.[1] Acute kidney injury (AKI) is considered an important determinant of mortality in patients undergoing cardiovascular surgery.[2] [3] Various epidemiological studies show that cardiac surgery-associated AKI (CS-AKI) occurs in up to 30% of cardiac surgery patients [4] and is associated with an increased morbidity as well as short- and long-term mortality.[5-8] The short-term and long-term mortality rate are 10.7% and 30% respectively and increase along with severity of stages.[6] Heart failure, chronic hyperglycemia, anemia, obesity, preoperative exposure to nephrotoxic drugs or contrast media, inflammation, proteinuria, and pre-existing kidney disease were systematically reviewed and were found to be associated with an increased risk of postoperative CS-AKI.[9]

In clinical studies evaluating potential treatments to prevent CS-AKI in high-risk groups, event rates of AKI up to 65.5% in a high-risk population [10] and 71.7% in patients with a positive biomarker test (that identifies patients at very high risk for AKI) shortly after surgery [11] have been reported. Recent studies on CS-AKI showed that patient enrichment based on the combination of the complexity of surgery and co-morbidities resulted in AKI rates between 28 and 34.2%.[12 13]

Current treatment strategies and clinical management of CS-AKI are mainly supportive, including optimal fluid management and maintenance of hemodynamic stability.[14] At present, no specific therapeutic interventions are available for prevention or treatment of CS-AKI. This is an area of large unmet clinical need, where patients who develop CS-AKI are at risk for additional severe complications, including need for renal replacement therapy (RRT), development of chronic kidney disease (CKD) and mortality. Thus, a single AKI episode after cardiac surgery can lead to a significant burden of comorbidities, prolonged hospital stays, poor quality of life and high long-term costs.

Recombinant alpha-1-microglobulin (RMC-035)

The investigational medicinal product (IMP), RMC-035, contains the pharmacologically active protein RMC-035, which is a recombinant and modified variant of endogenous human alpha-1-microglobulin (A1M). It has four distinct molecular mechanisms, including heme binding, reductase activity, radical scavenging, and mitochondrial binding/protection.[15] Employing several antioxidative mechanisms, it protects cells and tissues from various forms of cell damage caused by oxidative stress, including free radicals and reactive oxygen species (ROS). [16-19]

RMC-035 has been evaluated in four Phase 1 clinical studies: single ascending dose and multiple ascending dose studies in healthy subjects, a renal impairment study, and a phase 1b

pharmacokinetic and safety study in cardiac surgery patients. Available clinical data indicate that RMC-035 is safe and generally well tolerated across study populations and support its continued development in CS-AKI.

The pharmacological characteristics of RMC-035 have are appropriate for the chosen indication of CS-AKI as it targetsmultiple critical disease pathways of CS-AKI, including ischemia-reperfusion injury (IRI), heme toxicity and mitochondrial dysfunction. Furthermore, RMC-035 is rapidly distributed to kidney proximal tubular cells (via glomerular filtration), resulting in high drug exposure in the cells where the primary injury occurs in AKI. It is administered as intravenous (IV) infusion with short systemic half-life which is ideal for hospitalized patients requiring specific renal protection.

Trial objectives

This is a Phase 2, randomized, placebo-controlled, double-blind, adaptive design, parallel group clinical study to evaluate the efficacy and safety of RMC-035 in subjects at high risk for AKI following open-chest cardiac surgery. The study is being conducted to provide proof-of-concept efficacy data and to guide the design of a subsequent pivotal study in this patient population. Based on an integrated assessment of available non-clinical and clinical data, RMC-035 is considered to be well tolerated and is expected to provide targeted renal protection in this patient population, with the aim to prevent or ameliorate peri-operative and post-surgery renal injury.

Primary Objectives are to evaluate RMC-035 for the following outcomes:

• Prevention of AKI (Kidney Disease Improving Global Outcomes [KDIGO] definition [20]) in subjects undergoing cardiac surgery with additional risk factors for developing CS-AKI

• Safety and tolerability

Key secondary objectives are to evaluate RMC-035 for the following outcomes:

• To evaluate RMC-035 for the prevention of post-operative decline (within 72 hours) in renal function

• To evaluate RMC-035 for the reduction of post-operative AKI duration

Other secondary objectives are to evaluate RMC-035 for the following outcomes:

• To evaluate RMC-035 for preserving post-surgery renal function up to Day 90

• To evaluate RMC-035 for the prevention of post-operative dialysis up to Day 90

• To evaluate RMC-035 for the prevention of major adverse kidney events (MAKE) at Days 30 and 90, respectively

• To evaluate RMC-035 for the prevention of AKI within 72 hours (based on cystatin C and/or Urine Output [UO])

• To further evaluate RMC-035 for the prevention, persistence and severity of AKI within 72 hours (based on serum creatinine [SCr]

and/or UO or cystatin C and/or UO)and within 7 days (based on SCr and/or UO or cystatin C and/or UO)

• To evaluate RMC-035 for reducing post-operative albuminuria and proteinuria up to Day 90

• To evaluate the pharmacokinetics of RMC-035

• Identification and characterization of anti-drug-antibodies (ADA) developed after intravenous administration of RMC-035

Exploratory objectives are to evaluate:

• Post-baseline changes in kidney and cardiac biomarkers

• Length of post-operative stay in Intensive Care Unit (ICU) and overall hospitalization time

• Health-related Quality of Life

Methods and analysis

Patients

The target patient population is adults undergoing open-chest cardiac surgery, i.e. CABG and/or valve surgery and/or aorta surgery with additional predisposing risk factors for CS-AKI. Subjects planned to undergo cardiac surgery will be systematically screened for eligibility. Informed consent will be obtained by study site's principal or sub-investigators in all eligible patients before randomization and surgery. Inclusion and exclusion criteria are described in Table 1.

Table 1: Inclusion and Exclusion criteria

Inclusion criteria	A subject is eligible for the study if all of the following apply: 1. Institutional Review Board/ International Ethics Committee approved Informed Consent obtained 2. Ability to understand and comply with the study requirements and able to provide written informed consent 3. Age ≥18 and <85 years

	4. Estimated glomerular filtration rate (eGFR) is ≥30 mL/min/1.73 m2 5. Subject is scheduled for non-emergent coronary artery bypass grafting (CABG) surgery and/or valve surgery and/or ascending aorta aneurysm surgery with use of cardiopulmonary bypass (CPB), and AKI risk factors are present at screening 6. Female subject is not of child-bearing potential, or agreeing not to become pregnant 7. Female subject must not be breastfeeding 8. Female subject must not donate ova 9. Male subject and their female spouse/partner(s) who are of childbearing potential must be using a highly effective form of birth control 10. Male subjects must not donate sperm 11. Subject agrees not to participate in another interventional study
Exclusion criteria	Subject will be excluded from participation if any of the following apply: 1. Medical condition that makes the subject unsuitable for study participation 2. Scheduled for emergent surgeries (eg, aortic dissection) 3. Scheduled for CABG and/or valve surgery and/or ascending aorta aneurysm surgery combined with additional non-emergent cardiac surgeries (eg, congenital heart defects) 4. Scheduled to undergo transcatheter aortic valve implantation (TAVI) or transcatheter aortic valve replacement (TAVR), or off-pump surgeries or left ventricular assist device (LVAD) implantation 5. Experiences a cardiogenic shock or hemodynamic instability which require inotropes or vasopressors or other mechanical devices within 24 hours prior to surgery 6. Requirement for defibrillator or permanent pacemaker, mechanical ventilation, intraaortic balloon pumping (IABP), LVAD, or other forms of mechanical circulatory support (MCS) 7. Diagnosed with AKI (as defined by KDIGO criteria) within 3 months prior to surgery

	8. Required cardiopulmonary resuscitation within 14 days prior to cardiac surgery 9. Ongoing sepsis or an untreated diagnosed clinically significant infection (viral or bacterial) 10. Total bilirubin or alanine aminotransferase (ALT) or aspartate aminotransferase (AST) ≥ 2 times the upper limit of normal (ULN) 11. History of solid organ transplantation 12. History of renal replacement therapy (RRT) 13. Medical condition which requires active immunosuppressive treatment 14. Ongoing chemotherapy or radiation therapy for malignancy that may have an impact on kidney function 15. Received an investigational medicinal product within the last 90 days (or within 5 half-lives of the investigational drug, whichever is longer) 16. Subject has a known allergy to RMC-035 or one of its constituents, or has previously received RMC-035

Main outcomes

Primary Endpoints

Primary Efficacy Endpoint

Proportion of patients developing AKI within 72 hours after first dose of IMP, based on SCr and/or UO (AKI of any stage/severity according to KDIGO definition, ie, SCr ≥1.5 times baseline, or increase of SCr of ≥0.3 mg/dL [≥26.5 µmol/L], or UO <0.5 mL/kg/h for ≥6 hours).

Primary Safety Endpoint

Nature, frequency and severity of treatment-emergent adverse events (TEAEs; defined as any AE which occur within 72 hours after last IMP administration).

Secondary Endpoints

Key Secondary Endpoints

Time-corrected area under the curve (AUC) of SCr for Day 1 to Day 4 (72 hours after first dose of IMP)

Duration of AKI defined as the number of days meeting the definition of AKI (KDIGO definition) starting within 72 hours after first dose of IMP until resolution.

For other Secondary Endpoints and Exploratory Endpoints, please see Table 2.

Table 2: Other Secondary Endpoints and Exploratory Endpoints

Other Secondary Endpoints	- Post-baseline changes in renal function
	  - SCr and cystatin C (and corresponding eGFR values) at 12, 24,48, and 72 hours, respectively, and at Day 7/discharge, Day 30 and Day 90
	  - Change from baseline, up to Day 7/discharge, of peak SCr and cystatin C
	- Time-corrected AUC of cystatin C for Day 1 to Day 4 (72 hours after first dose of IMP)
	- Need for renal replacement therapy
	  - Dialysis treatment (for any reason) within 72 hours and within 7 days after end of surgery
	  - Dialysis free days from end of surgery to Day 30 and Day 90, respectively
	- MAKE at Day 30 and Day 90, defined as death, any dialysis, or ≥25% reduction of eGFR compared to baseline Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation (either SCr, cystatin C, or both)[21-23]
	- AKI Characteristics
	  - AKI within 72 hours after first dose of IMP based on cystatin C and/or UO (AKI of any stage/severity defined as cystatin C ≥1.5 baseline, OR UO <0.5 mL/kg/h for ≥6 hours)
	  - AKI within 7 days after first dose of IMP (based on SCr and/or UO criteria, or cystatin C and/or UO criteria)
	  - AKI persistence, defined as an AKI (KDIGO definition) developing within 72 hours after first dose of IMP and with a duration of ≥72 hours. Persistence will also be assessed per AKI severity stage*
	  - AKI severity stage* within 72 hours and within 7 days after first dose of IMP
	  - *Severity of AKI defined as the following:
	  -Stage 1: SCr 1.5 to 1.9 times baseline within 7 days, OR ≥0.3 mg/dL (≥26.5 µmol/L), OR urine output <0.5 mL/kg/h

	for 6 to <12 hours -Stage 2: SCr 2.0-2.9 times baseline within 7 days OR urine output <0.5 mL/kg/h for ≥12 hours -Stage 3: SCr 3.0 times baseline within 7 days, OR increase in SCr 4.0 mg/dL (≥353.6 µmol/L), OR initiation of renal replacement therapy OR urine output <0.3 mL/kg/h for ≥24 hours OR anuria for ≥12 hours
Exploratory Endpoints	- Post-baseline changes in urine albumin to creatinine ratio (UACR) and urine protein to creatinine ratio (UPCR) at Day 4, Day 30, and Day 90 - Pharmacokinetics of RMC-035 in plasma (AUC and Cmax) - Presence and titers of ADA at Day 1 (pre-surgery), Day 30, and Day 90 - Characteristics of ADA developed at Day 30 and Day 90 with regards to isotype, neutralizing capacity, and cross-reactivity with endogenous alpha-1-microglobulin (A1M) **Post-baseline changes in kidney and cardiac biomarkers** - Kidney biomarkers: Urine kidney injury molecule 1 (KIM-1), neutrophil gelatinase-associated lipocalin (NGAL), tissue inhibitor of metalloproteinase 2 (TIMP2), insulin like growth binding factor protein 7 (IGFBP7), chemokine ligand 14 (CCL-14), interleukin-18 (IL-18), liver fatty acid binding protein (LFABP) and 8-hydroxy-2'-deoxyguanosine (8-OHdG) - Cardiac Biomarkers: Plasma N-terminal-pro-hormone BNP (NT-pro BNP) and cardiac troponin I and T (cTnI, cTnT) **Hospitalization time and discharge facility** - Length of index ICU stay and index hospital stay - Index ICU stay (in Days) defined as the duration of stay in the ICU Immediately following surgery or recovery room post-surgery until ICU discharge - Index hospital stay (in Days) is defined as the duration of stay in the hospital from the day of surgery to hospital discharge for the index surgery - Nature of subject discharge facility (eg, home, skilled nursing facility, Rehabilitation center) **Health-related Quality of Life assessments** - Change from baseline to Day 90 in the following Patient Reported

	Outcomes (PROs): - MOS 36-Item Short Form Survey Instrument (SF-36) - European Quality of Life 5 Domain 5-Level Score (EQ-5D-5L)

Trial oversight

A scientific advisory board of experts in anesthesiology and cardiac surgery has been established by the sponsor to facilitate design of the trial, provide medical scientific leadership and make trial-specific recommendations to the sponsor. Likewise, an independent unblinded data monitoring committee (DMC) consisting of experts in acute kidney injury and a statistician will perform review of safety findings at regular intervals during the study. The working procedures of the DMC are defined in a DMC charter; their safety evaluation will be based on all available accumulated safety data. The DMC will also be responsible for recommendation on the study conduct following the interim analysis (IA) based upon predefined unblinded statistical output. Communication and recommendations by the DMC will be blinded. Completeness and accuracy of the data will be monitored by a Clinical Research Organization which performs regular monitoring visits.

Patient and public involvement statement

Reflecting the acute and unpredictable nature of CS-AKI there are no specific patient advocacy groups at present; closest are organizations that represent kidney diseases after cardiac surgery in general and, although they are aware of our program, they were not specifically consulted on the design of this study.

Trial design

Overall study design

This is a Phase 2, multi-center, randomized, double-blind, placebo-controlled, adaptive design, parallel group trial including patients undergoing open-chest cardiac surgery. The study consists of a screening period of up to 30 days before surgery, five in-hospital visits during the anticipated post-operative hospitalization period, and two additional follow-up visits at Day 30 and Day 90, respectively. A study flow chart is shown in Figure 1 and the schedule of study specific assessments is presented in Table 3. The study reporting was assessed following SPIRIT guidelines and is presented in the SPIRIT checklist in the supplementary material.

Table 3: Schedule of Assessments – Complete Study Protocol

Assessments	**Screening**	**Treatment**							**Follow-Up**			
Visit Number	**1** [h]	**2 Day of Surgery**					**3**	**4 (EOT)**	**5** [u]	**6 (discharge)** [v]	**7** [w]	**8 (EoS)** [w, x]
Visit Day	**-30 to -1**	**1**					**2 (24h)**	**3 (48h)**	**4 (72h)**	**7**	**30**	**90**
Allowed visit window (days)	**±0**	**±0**					**±0**	**±0**	**±0** [q]	**±2**	**±3**	**±7**
Visit hour (h)		pre	0h	1h	2h	6h [y]	12 h [y]					
Informed consent	x											
Inclusion/exclusion criteria	x	x										
Medical history	x											
Demographics	x											
Weight and height [a]	x						x	x	x			
Physical examination [b]	x								x			
Pregnancy test (WOCBP only) [c]	x	x										
Hematology lab [d]	x						x	x	x			
Clinical chemistry lab [d]	x						x	x	x			
Liver function lab [d]	x						x	x	x			
Serum creatinine (SCr) [e]	x	x					x	x	x	x	x	x
Serum Cystatin C [f]	x	x					x	x	x	x	x	x
UACR and UPCR [g]	x	x							x		x	x
Urinalysis		x							x			
Randomization [h]	x											
Record renal replacement therapy							x	x	x		x	x
IMP administration [i]			x [j]			x [k]	x [l]	x [l]	x [l]			
Plasma PK sampling		x		x	x			x, x, x [m]	x, x, x [m]			
12-lead electrocardiogram	x	x						x	x	x		
Vital signs [n]	x	x						x	x	x		
Surgery assessments [o]			←	—	—	→						
Discharge from ICU [p]							←	—	—	—	—	→

Urine output [q]		←										→
Urine sampling for biomarkers [r]		x				x		x	x				
Plasma/serum sampling for biomarkers [r]		x						x		x			
HRQoL Assessments [s]	x												x
ADA assessment		x										x	x
Concomitant medication recording [t]	←												→
AE recording				←									→
SAE recording		←											→

Abbreviations: ADA = anti-drug antibody; AE = adverse event; AKI = acute kidney injury; EOS = End of Study; EOT = End-of-Treatment; EQ-5D-5L = European Quality of Life 5 Domain 5-Level Score; HRQoL = health related quality of life; ICU = intensive care unit; IMP = investigational medicinal product; PK = pharmacokinetic; PRO = patient reported outcome; SAE = serious adverse event; SCr = serum creatinine; WOCBP = woman of childbearing potential; UACR = urine albumin to creatinine ratio; UAPR = urine albumin to protein ratio

a. Height only measured at screening (Visit 1). Weight during ICU stay only required if possible.

b. The initial physical examination performed at screening should be comprehensive; all other physical examinations may be abbreviated and symptom driven.

c. A serum pregnancy test completed during the screening period within 48 hours prior to surgery does not need to be repeated on the day of surgery. If the serum pregnancy test occurs more than 48 hours prior to the date of surgery, a serum or urine pregnancy test will also be performed on Day 1 prior to surgery.

d. Hematology Labs: Hematocrit, Hemoglobin (Hb), Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration, Red cell distribution width, Red blood cells, Platelets, Leucocytes (including Neutrophils, Monocytes, Lymphocytes, Eosinophils, Basophils)
Clinical Chemistry Labs: Albumin, Calcium, Chloride, Serum creatinine (SCr), C-reactive protein (CRP), Sodium, estimated glomerular filtration rate (eGFR), Magnesium, Phosphate, Potassium, Blood urea nitrogen (BUN), Uric acid, Glucose
Liver Function Labs: Alanine aminotransferase (ALAT), Alkaline phosphatase (ALP), Aspartate aminotransferase (AST), Bilirubin (total and conjugated), Gamma glutamyltransferase (GGT)

e. The screening sample for SCr must be collected on Day -1 (or day of surgery, see **footnote h**) and will be analyzed locally (to evaluate eligibility and determine correct start dose of RMC-035 according to renal function) and centrally (as baseline for endpoint assessment). All SCr samples collected during hospital stay will be analyzed both locally (to support AKI evaluation) and in a central lab (for the purpose of endpoint assessments). Day 30 and 90 samples will be analyzed centrally.

f. Cystatin C samples will be collected and analyzed in a central lab only for the purpose of endpoint assessments

g. UACR: screening sample will be collected as a spot urine sample and analyzed locally to evaluate albuminuria as an eligibility criterion (in the absence of historical albuminuria data within 3 months prior to randomization). UACR and UPCR: In-hospital samples (Day 1, Visit 2 and Day 4, Visit 5) will be collected either as a First Morning Void (FMV) sample or drawn directly from a Foley catheter and analyzed in a central lab only. Follow-up samples (Day 30, Visit 7 and Day 90, Visit 8) will be collected as FMV samples as possible and analyzed in a central lab only.

h. Randomization must occur on Day -1, ie the day before surgery is intended. All screening assessments may be performed on Day 1 prior to surgery, including randomization. These assessments must be completed prior to any pre-surgical activities, such as administration of fluids or medications, including anesthesia.

i. All 5 doses to be calculated using the same weight measurement that is used for randomization / stratification. IMP will be permanently discontinued in subjects developing AKI stage 2 or higher as per KDIGO guidelines

j. IV infusion over 60 minutes, first infusion should start approximately 10 minutes before expected onset of CPB (time point 0 is defined as start of IMP administration)

k. IV infusion over 60 minutes at 6 h (±30 min) after the start of first infusion

l. IV infusion over 30 minutes at 12 h, 24 h and 48 h (±30 min) after the start of first infusion

m. PK sampling at Day 2 and 3 should occur 30 min (±5 min) and 90 min (±15 min) from start of IMP infusion

Plasma PK Sampling Study Day	Time Window	**Predose** ≤30 min	**30 min** ±5 min	**1 h** ±5 min	**90 min** ±15 min	**2 h** ±15 min
Day 1	Start of Infusion 1 (t=0 h)	x		x		x
Day 2	Start of Infusion 4 (t=24 h)	x	x		x	
Day 3	Start of Infusion 5 (t=48 h)	x	x		x	

n. Vital signs: body temperature, blood pressure, heart rate, respiratory rate, SpO2

o. Data points to collect are type of CPB pump (pulsatile or non-pulsatile, if applicable) and duration of CPB (exact time of initiation and end of CPB), duration of surgery (beginning of surgery defined as exact time of initial skin incision, end of surgery defined as exact time of skin closure), blood loss volume, administration of any fluids during surgery (blood products [red blood cells, plasma, cryoprecipitate, platelets, etc.], crystalloids, colloids, and others), target body temperature during CPB and time at temperature range, duration of cross clamp (minutes), number, position, and graft source bypasses performed, length of time with mean arterial pressure <50 mmHg, valve surgery type (replacement or repair), replacement valve origin (bioprosthetic or mechanical), aortic repair type, and time of admission to the ICU.

p. Time of discharge from ICU to hospital ward, another treatment facility or home

q. Only required as long as Foley catheter is in place

r. Urine samples for evaluation of exploratory urinary (kidney) biomarkers and plasma/serum samples for evaluation of cardiac biomarkers will be collected at the intervals described below, respectively:

Biomarker Sampling Time Window (in relation to Start of Infusion 1 (t=0 h))	**Predose** ≤60 min	**6 h** ±30 min	**24 h** ±30 min	**48 h** ±30 min	**72 h** ±30 min
Urine	x	x	x	x	
Plasma/serum	x		x		x

s. PRO HRQoL assessment: SF 36 and EQ-5D-5L. PRO HRQoL assessments to be performed as early as possible in the screening period.

t. Medications taken within 30 days prior to the day surgery is intended are to be collected. Use of contrast agent within 72 hours prior to the day surgery is intended should be documented as a prior/concomitant medication. When possible, type and quantity of contrast agent should be recorded.

u. Visit 5 must occur at 72 hours from start of first infusion of IMP, with a scheduling window of +/-2 hours.

v. Visit 6 and all associated assessments should occur on the day of hospital discharge. In case subject is discharged on Day 4, discharge (Visit 6) assessments performed prior to discharge on that day are acceptable.

w. Visit may be performed by qualified and trained study staff at the subject's home or other suitable location, where appropriate

x. In case of subject withdrawal, subject should be encouraged to undergo all EOS assessments as an Early Termination visit.

y. Assessments must be performed prior to IMP administration

Randomization procedure

The trial population consists of patients at high risk for AKI and undergo cardiac surgery with additional predisposing risk factors for CS-AKI (inclusion and exclusion criteria are shown in Table 1). Eligible subjects will be randomized to receive either RMC-035 or placebo in a 1:1 randomization ratio. Both region (EU versus North America) and pre-operative eGFR calculated using the CKD-EPI equation with SCr on local laboratory results (≥60 and <60 mL/min/1.73m^2) will be used as stratification factors to ensure a balanced randomization within these groups. Randomization will be performed by the investigators using the centralized electronic randomization platform. Randomization codes will be subsequently computer-generated and sent to study pharmacists for IMP preparation.

IMP presentation and blinding

The Investigator, study personnel, and subject will be blinded to the identity of the IMP (RMC-035 or placebo). The investigational pharmacist will be responsible for the preparation of IMP for each subject and will be unblinded to the randomization assignment. In the event of a medical emergency requiring knowledge of the treatment assigned to the subject, unblinding can be performed and the treatment code for a given randomized subject will be provided. The time, date, subject number and reason for obtaining any of these codes, and therefore breaking the blind, will be documented in the study file. The treatment code can only be requested by the Investigator or other persons designated as sub-investigators. No subjects or study personnel will be made aware of the treatment given unless a medical emergency necessitates such disclosure. Both IMP and placebo will be presented in 50ml syringes and labelled in a standardized manner. Label information includes study protocol number, subject-ID, syringe number, date and time of IMP preparation, IMP content (RMC-035 or placebo) and sponsor contact information, but no information about randomization group. Both RMC-035 and placebo are transparent, odorless fluids that cannot be differentiated optically or by other sensations. Pharmacokinetic samples taken at the study sites and evaluated in a central laboratory of the sponsor will be used to determine systemic exposure to study drug.

IMP dosing

In total five IV doses of either RMC-035 or placebo will be administered during the study. The first dose will start approximately 10 minutes before cardiopulmonary surgery is initiated (0h), and subsequent doses will be given at 6, 12, 24, and 48 hours later. IMP will be given as a continuous IV infusion over either 60 minutes (first two doses) or 30 minutes for subsequent doses. Due to the pharmacokinetic properties of RMC-035, the predefined dose levels (prior to surgery) are based on renal function (eGFR by CKD-EPI equation with SCr) at screening (Day -1, Visit 1): 1) subjects with eGFR ≥60 mL/min/1.73m^2 will receive 1.3 mg/kg (per dose) for the first and second dose, followed by 0.65 mg/kg (per dose) for the third, fourth and fifth

dose; 2) Subjects with eGFR >30 and <60 mL/min/1.73m^2 will receive 0.65 mg/kg (per dose) for all five doses. The trial drug is provided on top of standard of care of each center.

Permanent discontinuation of IMP

Local laboratory results will be utilized for clinical care and real time evaluation of AKI for purposes of IMP discontinuation. Subjects who meet any of the following IMP discontinuation criteria during the treatment period will be discontinued from IMP and continue study participation and procedures and follow up as per the schedule of assessment until the end of the study visit: 1) Development of stage ≥2 AKI, according to KDIGO definition, 2) Need for RRT, 3) Need for percutaneous or surgical mechanical circulatory support (MCS) or extra-corporeal membrane oxygenator (ECMO), 4) Reporting of a grade 3 (per Common Terminology Criteria for Adverse Events [CTCAE]) or higher adverse event of ISR or IRR, and which is considered to be an immune-mediated reaction

The IMP will be discontinued in case of any of the following abnormal liver chemistry tests in blood: 1) ALT > 3 × ULN combined with total bilirubin > 2 × ULN in the same sample, 2) ALT > 3 × ULN if associated with symptoms (new or worsening) believed to be related to hepatitis (such as fatigue, nausea, vomiting, right upper quadrant pain or tenderness or jaundice) or believed to be related to hypersensitivity (such as fever, rash or eosinophilia), 3) ALT > 3 x ULN if confirmed in a second sample within 24 hours AND in the second sample International Normalized Ratio (INR) is increased to >1.5 x ULN, 4) ALT > 8 x ULN in any individual sample during the treatment period All liver chemistry abnormalities as summarized above need to be followed up regularly (at least every 24 hours) until values have returned to baseline or are considered stable. If IMP is discontinued, the subject will remain in the study to be evaluated until the end of the study Visit (as much as possible) or complete the end of the study visit assessments.

Planned sample size & interim analysis (IA)

A minimum of 268 and a maximum of 348 patients are planned to be randomized at approximately 30 sites predominantly across Europe and North America. The final number of patients randomized will depend on the results of the IA.

There will be one IA during the trial which will be conducted once 50% of the planned randomized subjects have completed visit 6 (Day 5-9). Sample size may be increased to a maximum of 348 randomized subject but will not be decreased. The study may be stopped at the IA for futility or safety reasons. Study eligibility criteria may also be modified.

Safety reporting

Safety is followed up until last study visit at Day 90. Serious adverse events (SAEs) will be reported to the sponsor and other stakeholders as per regulatory requirements (eg, competent authorities and/or IRBs/CECs, when applicable). Non-serious AEs will only be reported up to Day 30 Treatment emergent AEs (TEAEs) are defined as AEs which occur after the initial IMP administration through 72 hours after last IMP administration. Safety reporting will be managed by a clinical research organization in compliance with regulatory requirements of each region of the study.

Statistical Considerations

For the primary endpoint, KDIGO AKI (serum creatinine and urine output) within 72 hours after first dose of IMP, RMC-035 has been assumed to lead to a 30% relative risk reduction vs. placebo. The event rate in the placebo group has been assumed to be 50%.[24] A sample size of 268 subjects randomized leads to a test power of 80% to show statistically significant results at a two-sided significance level (alpha) of 0.10.

The primary endpoint will be analyzed by the Cochran-Mantel-Haenszel estimate of the common relative risk (RMC-035 vs. placebo) across the four stratification groups formed by region and pre-operative eGFR (≥60 and <60 mL/min/1.73m2). In addition, the proportion of subjects with AKI within 72 hours after first dose of IMP and its 90% confidence interval will be calculated for each treatment group. The hypotheses to be tested are as follows: 1) Null hypothesis: the proportion of subjects developing AKI within 72 hours after first dose of IMP is the same for the RMC-035 and the placebo treatment groups ($\mathbf{p}_{\text{RMC-035}} = \mathbf{p}_{\text{placebo}}$), 2) Alternative hypothesis: the proportion of subjects developing AKI within 72 hours after first dose of IMP is different for the RMC-035 and the placebo treatment groups ($\mathbf{p}_{\text{RMC-035}} \neq \mathbf{p}_{\text{placebo}}$).

All secondary endpoints have a supplementary character and will be reported with no strict control of the type I error. Secondary continuous efficacy endpoints that are measures of renal function will be analyzed using robust regression of log-transformed values having sequentially imputed any missing data using multiple imputation. A sensitivity analysis will be performed using a mixed model for repeated measurements (MMRM) if data are approximately normally distributed.

For the key secondary AUC endpoint, the geometric least square means of the time-corrected AUC of SCr for Days 1 to 4 will be obtained for each, RMC-035 and placebo, by transforming the model estimates back to the original scale.

Supportive analyses of renal function will be performed on cystatin C levels (and corresponding eGFR values assessed by Chronic Kidney Disease Epidemiology Collaboration [CKD-EPI] equations with either SCr, cystatin C, or both) and cumulative AUC values up to each timepoint. Secondary binary efficacy endpoints will be analyzed using the same approach as specified above for the primary endpoint.

Further details on the IA and the control of the type I error will be provided in the DMC Charter and the Statistical Analysis Plan (SAP).

Safety Endpoints

Safety analyses will be conducted on the Safety Analysis Set (SAF), which includes all randomized subjects who received at least 1 dose of IMP. To characterize the safety profile, the number and percentage of subjects with TEAEs and AEs per dose group will be tabulated. Descriptive statistics will be provided for laboratory tests (hematology, biochemistry, urinalysis) and vital signs (pulse rate, respiratory rate and blood pressure) by visit and for the changes from baseline to each visit split by treatment group. In addition, the development of anti-drug antibodies after intravenous administration of RMC-035 will be investigated and characterized.

Pharmacokinetics

Descriptive statistics will be presented for plasma concentrations by scheduled sample time. Specific pharmacokinetic parameters will be presented separately. Summaries will be provided by dose group, age group and by renal function prior to surgery.

Sensitivity analyses and secondary endpoints

Secondary continuous efficacy endpoints that are measures of renal function will be analyzed using robust regression of log-transformed values having sequentially imputed any missing data using multiple imputation. A sensitivity analysis will be performed using a mixed model for repeated measurements (MMRM) if data are approximately normally distributed.

For the key secondary AUC endpoint, the geometric least square means of the time-corrected AUC of SCr for Days 1 to 4 will be obtained for each, RMC-035 and placebo, by transforming the model estimates back to the original scale. The relative difference (RMC-035 vs. placebo) and its 90% confidence interval will also be reported.

Supportive analyses of renal function will be performed by using cystatin C levels (and corresponding eGFR values assessed by Chronic Kidney Disease Epidemiology Collaboration [CKD-EPI] equations with either SCr, cystatin C, or both) and cumulative AUC values up to each timepoint.

Secondary binary efficacy endpoints will be analyzed using the same approach as specified above for the primary endpoint.

Additional sensitivity analyses of the primary and secondary endpoints, and evaluation of exploratory endpoints, will be detailed in the SAP.

Ethics and dissemination

This trial will be conducted in accordance with the protocol and consensus ethical principles derived from international guidelines including the Declaration of Helsinki and Council for International Organizations of Medical Sciences (CIOMS) International Ethical Guidelines; applicable International Conference on Harmonization (ICH) GCP Guidelines; and applicable laws and regulations. The protocol, substantial protocol amendments, ICF, IB, and other relevant documents (e.g., any other written information regarding this trial to be provided to the patient or the patient's legal representative) are approved by the Ethics Committee of the Physicians Chamber Westfalen-Lippe and the University of Münster in Germany (file number 2021-778-f-A).

Informed consent will be given by patients before any study relevant procedure is performed. Patients will be assigned a unique patient identification number. Any patient records or datasets that are transferred to the Sponsor will contain this identifier only; patient names and any information which would make the patient identifiable will not be transferred. All laboratory specimens, evaluation forms, reports, and other records will be identified in a manner designed to maintain patient confidentiality.

Irrespective of whether the trial is completed or prematurely terminated, the sponsor will ensure that the trial results will be posted on publicly available clinical trial registries in accordance with their requirements. In addition, results will be presented at international congresses and published in peer-reviewed journals. Participant-level access to data will not be granted to the public.

Conclusions

The ongoing Phase 2 (AKITA) study is specifically designed to answer the primary research question whether the investigational drug RMC-035 can reduce the occurrence of CS-AKI within 3 days after open cardiac surgery. Study results will also provide pertinent information on other clinically relevant endpoints, such as the severity, duration and persistence of AKI, changes in post-operative renal function and major adverse kidney events (MAKE), and will significantly expand the current knowledge of the safety profile of RMC-035. Finally, biomarker analysis may improve the understanding of its mechanism of action, shed light on relevant disease pathways in CS-AKI and facilitate the design of future clinical trials of RMC-035.

Author's contributions

AZ, CDM, TEL, and RR co-drafted the manuscript. NSF, JB, FW, AM, CCDB, JLK, AB, DTE, BdV, NN, MT, AL, ML, TvG and CR participated in trial protocol design, reviewed and edited the manuscript.

Funding statement

This work was supported by Guard Therapeutics.

Competing interest statement

Alexander Zarbock received advisory board consultancy and travel reimbursements from Guard Therapeutics.

Data availability statement: Data obtained during the trial is the property of the study sponsor and will be handled in compliance with data protection policies and regulations.

Word count:

3273 words

Figure legend

Figure 1: study flowchart

Ethics Approval

The study was approved by the Ethics Committee of the Physicians Chamber Westfalen-Lippe and the University of Münster in Germany (file number 2021-778-f-A).

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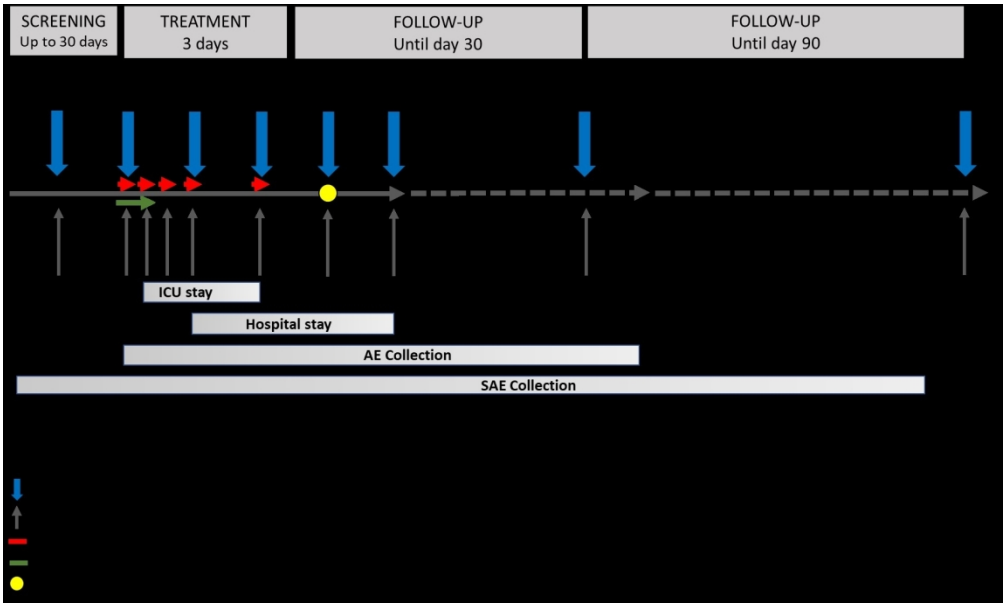

Figure 1: Study flowchart

307x183mm (150 x 150 DPI)

Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

In your methods section, say that you used the SPIRITreporting guidelines, and cite them as:

Chan A-W, Tetzlaff JM, Gøtzsche PC, Altman DG, Mann H, Berlin J, Dickersin K, Hróbjartsson A, Schulz KF, Parulekar WR, Krleža-Jerić K, Laupacis A, Moher D. SPIRIT 2013 Explanation and Elaboration: Guidance for protocols of clinical trials. BMJ. 2013;346:e7586

		Reporting Item	Page Number
Administrative information			
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
11Trial registration	#2a	Trial identifier and registry name. If not yet registered, name of intended registry	2
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	2
Protocol version	#3	Date and version identifier	n/a
Funding	#4	Sources and types of financial, material, and other support	12
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	1
Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	1
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication,	12

		including whether they will have ultimate authority over any of these activities	
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	7
Introduction			
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	4,5
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	n/a
Objectives	#7	Specific objectives or hypotheses	5,6,7
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	8-11
Methods: Participants, interventions, and outcomes			
Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	9
Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	6, Table 1
Interventions:	#11a	Interventions for each group with sufficient detail to	7,8

description		allow replication, including how and when they will be administered	
Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	8,9
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	8,11
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	Table 1
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	5-7
Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	Table 3
Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	9
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	9
Methods: Assignment of interventions (for controlled trials)			
Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of	8

		any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	
Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	8
8Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	8
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	8
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	8
Methods: Data collection, management, and analysis			
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	10,11
Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	7

Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	7
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	10-12
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	11,12
Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	11,12
Methods: Monitoring			
Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	7
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	10
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	10
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent	10

		from investigators and the sponsor	
Ethics and dissemination			
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	2,12
Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	n/a
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	6,12
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	n/a
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	7,12
Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	13
Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	13
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	n/a
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	12

Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	n/a
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	12
Appendices			
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	Supplementary material
Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	n/a

CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	1
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	2
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	4,5
	2b	Specific objectives or hypotheses	6
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	6-11
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	6-11
Participants	4a	Eligibility criteria for participants	6,Table 1, Table 3
	4b	Settings and locations where the data were collected	1
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	8,9
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	6,7,Table 2
	6b	Any changes to trial outcomes after the trial commenced, with reasons	-
Sample size	7a	How sample size was determined	9,10
	7b	When applicable, explanation of any interim analyses and stopping guidelines	9,10
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	8
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	8
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	8
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	8

Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those assessing outcomes) and how	7,8
	11b	If relevant, description of the similarity of interventions	-
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	11-12
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	10
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	-
	13b	For each group, losses and exclusions after randomisation, together with reasons	-
Recruitment	14a	Dates defining the periods of recruitment and follow-up	-
	14b	Why the trial ended or was stopped	-
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	-
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	-
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	-
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	-
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	-
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	-
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	3
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	3
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	-
Other information			
Registration	23	Registration number and name of trial registry	2
Protocol	24	Where the full trial protocol can be accessed, if available	-
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	16

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.

SPIRIT
STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Page no.
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	2
	2b	All items from the World Health Organization Trial Registration Data Set	2
Protocol version	3	Date and version identifier	n/a
Funding	4	Sources and types of financial, material, and other support	20
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1
	5b	Name and contact information for the trial sponsor	20
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	20
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	11
Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	4-5
	6b	Explanation for choice of comparators	5
Objectives	7	Specific objectives or hypotheses	5-6

Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	11-12
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	1
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	6-8
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	11-16
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	16-17
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	18
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	7-8
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	8-11
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	11-15
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	17-18
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	n/a
Methods: Assignment of interventions (for controlled trials)			
Allocation:			

Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	15
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	15
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	15
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	15-16
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	15-16
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	17-18
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	n/a
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	19
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	17-19
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	18-19
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	n/a

Methods: Monitoring

Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	11-17
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	11
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	17
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	17

Ethics and dissemination

Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	19
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	19
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	19
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	n/a
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	19
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	20
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	20
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	n/a

Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	19
	31b	Authorship eligibility guidelines and any intended use of professional writers	n/a
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	20
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Supplement
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	n/a

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.