CLINICAL STUDY PROTOCOL

Protocol Title:	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
Study Name	AKITA
Protocol Number:	21-ROS-05
Investigational Product:	RMC-035 or Placebo
EudraCT Number:	2021-004040-19
Clinical Study Sponsor:	Guard Therapeutics International AB Nybrogatan 34, SE-114 39 Stockholm, Sweden
Version:	3.1
Protocol Amendment Date	08 October 2022

SIGNATURE PAGE

Sponsor's Approval

The protocol has been approved by Guard Therapeutics International AB.

Sponsor Signatories:

Dr. med. [redacted]
Medical Lead / Chief Medical Officer

[redacted] MSc
Head of Clinical Operations

[redacted] MSc
Biostatistician

Lead Investigator Signatory:

Prof. Dr. med. [redacted]
Lead Investigator

INVESTIGATOR'S AGREEMENT

I have read the attached protocol 21-ROS-05 **Version 3.1** (amendment 3.0) titled: 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, and agree to abide by all provisions set forth therein.

I agree to comply with the International Conference on Harmonisation Tripartite Guideline on Good Clinical Practice and applicable national or regional regulations and guidelines.

I agree to ensure that the confidential information contained in this document will not be used for any purpose other than the conduct of the clinical investigation without prior written consent from Guard Therapeutics International AB.

_____

Signature Date

_____

Printed Name

EMERGENCY PERSONNEL

Table 1: Emergency Contact Information

Role in Study	Name	Contact Information
Medical Monitor / 24-Hour Emergency Contact	Robert Gaston, MD Sr. Medical Director CTI Clinical Trial & Consulting Services	100 E. RiverCenter Blvd Covington, KY 41011 Email: rgaston@ctifacts.com Phone: USA/Canada: 1 800 936 1593 Spain: 900 876 257 Germany: 0800 1806593 Czech Republic: 800 144 262
SAE Reporting	CTI Clinical Trial & Consulting Services	100 E. RiverCenter Blvd Covington, KY 41011 SAE eFax number: USA: 1 800 695 3418 Canada: 1 877 349 5952 Spain: 900 807 339 Germany: 7319 7929001 Czech Republic: 227 204 969 Toll Free SAE Phone Hotline: USA: 1-877-755-0742 Canada: 1 877 832 6358 Spain: 900 1817513 Germany: 0800 1817513 Czech Republic: 800 144 513

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LIST OF ABBREVIATIONS

Table 2: Abbreviations and Terms

Abbreviation or Specialist Term	Explanation
8-OHdG	8-hydroxy-2'-deoxyguanosine
A1M	Alpha-1-microglobulin
ACEi	Angiotensin-converting enzyme inhibitor
ADA	Anti-drug antibodies
ADL	Activities of daily living
AE	Adverse event
AKI	Acute kidney injury
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
ARB	Angiotensin receptor blocker
AST	Aspartate aminotransferase
AUC	Area under the curve
BPM	Beats per minute
CABG	Coronary artery bypass grafting
CCL-14	Chemokine ligand 14
CIOMS	Council for International Organizations of Medical Sciences
CKD	Chronic kidney disease
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration
C_{max}	Maximum (peak) concentration observed
CP	Conditional power
CPB	Cardiopulmonary bypass
CRF	Case report form
CRO	Contract research organization
CS-AKI	Cardiac surgery associated acute kidney injury
CSR	Clinical study report
DMC	Data monitoring committee
cTnI	Cardiac troponin I
cTnT	Cardiac troponin T

Abbreviation or Specialist Term	Explanation
CVC	Central venous catheter
ECG	Electrocardiogram
ECMO	Extra-corporeal membrane oxygenator
EDC	Electronic data capture
eGFR	Estimated glomerular filtration rate
EOS	End-of-study
EOT	End-of-treatment
EQ-5D-5L	European quality of life 5 domain 5-level score
FDA	Food and Drug Administration
FMV	First morning void
FSH	Follicle stimulating hormone
GCP	Good clinical practice
GLP	Good laboratory practice
h	Hour(s)
HRQoL	Health related quality of life
HRT	Hormone replacement therapy
IA	Interim analysis
IABP	Intra-aortic balloon counter-pulsation
IB	Investigator's brochure
ICF	Informed consent form
ICH	International Conference on Harmonization
ICU	Intensive care unit
IEC	Independent ethics committee
Ig	Immunoglobulin
IGFBP7	Insulin-like growth binding factor 7
IL-18	Interleukin-18
IMP	Investigational medicinal product
INR	International normalized ratio
IRB	Institutional review board
IRR	Infusion related reaction
ISR	Infusion site reaction
ITT	Intent to treat

Abbreviation or Specialist Term	Explanation
IUD	Intrauterine device
IUS	Intrauterine hormone releasing system
IV	Intravenous
KIM-1	Kidney injury molecule 1
LFABP	Liver fatty acid binding protein
LVAD	Left ventricular assist device
LVEF	Left ventricular ejection fraction
MAD	Multiple ascending dose
MAKE	Major adverse kidney events
MCS	Mechanical circulatory support
MedDRA	Medical Dictionary for Regulatory Activities
mITT	Modified intent to treat
MMRM	Mixed model for repeated measurements
MRI	Magnetic resonance imaging
NGAL	Neutrophil gelatinase-associated lipocalin
NOAEL	No observed adverse effect levels
NSAID	Non-steroidal anti-inflammatory drugs
NT-pro-BNP	N-terminal-pro-b-type natriuretic peptide
NYHA	New York Heart Association
PI	Principal Investigator
PK	Pharmacokinetic
PPS	Per protocol set
PRO	Patient reported outcome
PT	Preferred term
RRT	Renal replacement therapy
RAAS	Renin-angiotensin-aldosterone system
ROS	Reactive oxygen species
SAD	Single ascending dose
SAE	Serious adverse event
SAF	Safety analysis set
SAP	Statistical analysis plan
SAR	Serious adverse reaction

Abbreviation or Specialist Term	Explanation
SCr	Serum creatinine
SD	Standard deviation
SF-36	Medical outcomes study 36-item short form survey instrument
SOA	Schedule of assessments
SOC	System organ class
SOP	Standard operating procedure
SpO2	Oxygen saturation
SUSAR	Suspected unexpected serious adverse reaction
T2DM	Type 2 diabetes
TAVI	Transcatheter aortic valve implantation
TAVR	Transcatheter valve replacement
TEAE	Treatment emergent adverse event
TIMP2	Tissue inhibitor of metalloproteinase 2
TMF	Trial master file
UACR	Urine albumin-to-creatinine ratio
UPCR	Urine protein-to-creatinine ratio
ULN	Upper limit of normal
UO	Urine output
VAS	Visual analogue scale
WOCBP	Woman of childbearing potential

1. SYNOPSIS

Name of Sponsor/Company: Guard Therapeutics International AB		
Name of Investigational Product: RMC-035		
Indication: Cardiac Surgery Associated Acute Kidney Injury (CS-AKI)		
Protocol Number: 21-ROS-05	**Phase: 2**	**Regions:** North America, Europe
Title of Study: 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		
Studied period (years): Estimated date first subject enrolled: Quarter 4 2021 Estimated date last subject completed: Quarter 2 2023		

Objectives:

Primary Objectives:

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

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)

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

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 estimated glomerular filtration rate (eGFR) compared to baseline using the Chronic Kidney Disease-Epidemiology Collaboration (CKD-EPI) equations (with either SCr, cystatin C, or both)
- 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 C_{max})
- 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)

Changes in immunologic biomarkers

- Immunologic biomarkers to explore the background and mechanism of potential IRRs including but not limited to markers of complement activation, cytokine release and mast cell activation.

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

Methodology:

This is a Phase 2, randomized, double-blind, adaptive, parallel group clinical study that will evaluate RMC-035 compared to placebo with approximately 268 subjects at high risk for CS-AKI based on pre-defined risk factors. An interim analysis (IA), including a sample size re-assessment, will be performed during which subject enrollment will continue. The study will be performed at approximately 30 sites in Europe and North America and consists of 8 visits for each subject with four study periods:

- Screening Period: 30 days (Day -30 to -1)
- Treatment Period: 3 days (Day 1-3)
- Follow-Up Period: 27 days (Day 4-30)
- Extended Follow-Up Period: 60 days (Day 31-90)

See the Schedule of Assessments (SOA, Table 3) for the allowed time window for each study visit. During the course of the study, visits and assessments will be performed as defined in the SOA.

Screening Period and Randomization (Visit 1)

Prospective subjects may be consented within 30 days (inclusive) prior to the date of scheduled surgery. Subjects who have signed the informed consent may undergo initial screening assessments during the screening period (full physical examination, medical history, clinical laboratory testing, completion of health-related quality of life questionnaires etc.) as described in the SOA.

All screening clinical laboratory testing to confirm eligibility and determine starting dose based on renal function will be performed by local laboratory on Day -1 (Visit 1), ie, the day before surgery is intended. In addition, samples for serum creatinine and cystatin C will be collected for central laboratory analysis which will provide the baseline values for the purpose of relevant endpoint assessment.

Eligible subjects will be randomized on Day -1 (Visit 1) to receive either RMC-035 or placebo in a 1:1 randomization ratio. Both region (North America and Europe) and Day -1 eGFR calculated using 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.

Note: Some or all screening visit assessments listed in the SOA, including randomization, may be performed prior to surgery on Day 1 (Visit 2), ie, the day of surgery, if feasible for the site.

Note: In case surgery is postponed after randomization has occurred, re-assessment of SCr and body weight for the calculation of eGFR is not required. However, if re-assessment is done as part of standard of case, and the results indicate an eGFR decrease that places the subject in a different stratum (ie <60 mL/min/1.73m^2 for a subject who was randomized to the ≥60 mL/min/1.73m^2 stratum) a decrease of the IMP dose to that of the lower stratum is acceptable if the change is clinically significant in the opinion of the investigator.

Treatment Period (Visit 2-4)

Surgery will occur on Day 1 (Visit 2). Before administration of IMP, preoperative safety assessments and sampling for local and central clinical laboratory testing will be performed per the SOA. Centrally processed laboratory samples of morning urine collected pre-surgery on Day 1 will provide the baseline values for evaluation of relevant endpoints (UACR and UPCR).

The first administration of Investigational Medicinal Product (IMP: RMC-035 or Placebo) will be given during surgery, approximately 10 minutes before the expected initiation of cardiopulmonary bypass (CPB), as an intravenous (IV) infusion. The start time of the first infusion is time 0, the reference point for further dose administrations and protocol-mandated assessments. In total, 5 doses of IMP will be administered during the treatment period. Administration of subsequent IV infusions start at 6, 12, 24 and 48 hours, respectively, after time 0 (start of the first dose).

Administration of RMC-035 or placebo:

- The first (0h) and second (6h) doses are administered as IV infusions over 60 minutes
- The third (12h), fourth (24h) and fifth (48h) doses are administered as IV infusions over 30 minutes

Due to the pharmacokinetics 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):

- 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
- Subjects with eGFR >30 and <60 mL/min/1.73m^2 will receive 0.65 mg/kg (per dose) for all five doses

Differences between eGFR at Day -1 and additional eGFR calculations collected on Day 1 prior to surgery as part of the study or per the investigational site's standard of care will not affect eligibility and starting dose unless the change is a decrease that the investigator considers clinically significant and that implies the subjects should have a lower starting dose. Should the subject eGFR drop below 30 ml/min/1.73m^2, and in the investigator's opinion the decrease is clinically significant, the subject is no longer fulfilling eligibility criteria and should not start treatment. Discontinuation of IMP:

- Investigational Medicinal Product will be permanently discontinued in subjects developing AKI stage 2 or higher as per KDIGO guidelines and for other reasons as detailed in Section 5.3.1.

Investigational Medicinal Product should preferably be administered through a central venous catheter (CVC) in a lumen which is reserved for the IMP. If the CVC is removed during the treatment period, the IMP may be administered via a catheter in a peripheral vein. This catheter should preferably be reserved for IMP administration only.

Surgery assessments (specified in the SOA) performed before, during, and after surgery will be collected per standard of care and documented in the case report form (CRF). All medications administered before and during surgery, including anesthesia, will be recorded as concomitant medications. Postoperative admission of the subject to the ICU will also be recorded in the CRF.

Urine will be collected starting on Day 1 and will continue only for as long as the subject has a urinary catheter (Foley) in place to calculate urine output (UO), or until 72 hours after first dose of IMP (if catheter remains in place). Urine output (volume) will be entered into the CRF at minimum by 6-hour time periods up to a maximum of 72 hours after end-of-surgery; the exact time period for the collection and the collected volume will be recorded in the CRF.

If the Foley catheter is removed, urine production will no longer be documented in the CRF but urine samples collection will continue as per the SOA. UO can only be used for diagnosing AKI or rating

AKI severity if UO has been calculated based on UO measured by a Foley catheter. If the Foley catheter is removed, UO will not be used for the AKI definition.

IMP will be administered at 6, 12, 24 and 48 hours after start of the first dose administration. In case the subject is discharged from the ICU to a non-ICU ward prior to last dose administration, the remaining dose(s) will be administered either by study-trained non-ICU staff, or preferably by study-trained ICU staff at the non-ICU ward. Blood and urine samples will be collected every day during the treatment period as per SOA, ie, 24 hours after start of initial IMP infusion (Day 2, Visit 3) and 48 hours after start of initial IMP infusion (Day 3, Visit 4; End-of-Treatment [EOT]).

Follow Up Period (Visit 5-7)

After the final dose has been administered (Day 3, Visit 4, EOT), additional study visits will be conducted at 72 hours after first dose of IMP (Day 4, Visit 5, Primary endpoint assessment) and between Day 5-9 (Visit 6, Discharge) before hospital discharge where blood and spot urine samples will be taken as per SOA. When the subject is discharged from the ICU either to the hospital ward, another treatment facility or home, this will be recorded separately in the CRF.

After hospital discharge, subjects will initially be followed up for one month after surgery until the next study visit (Day 30, Visit 7). This visit may be performed by qualified and trained study staff at the investigational site, at the subject's home, or other suitable location, where appropriate. Blood and urine samples collected will be processed centrally. During this follow-up period, all AEs need to be reported to the Investigator as soon as possible, eg, by phone. The Investigator will decide whether a visit to the clinic or hospital needs to be scheduled.

Extended Follow Up Period (Visit 8)

A final study visit will be conducted at 3 months (Day 90, Visit 8, End of Study [EOS]). This visit may be performed by qualified and trained study staff at the investigational site, at subject's home, or other suitable location, where appropriate. Health related quality of life questionnaires will be collected. Blood and urine samples collected will be processed centrally. Subjects are requested to provide information about hospitalization periods and other events qualifying as serious adverse events (SAEs). Vital status (ie, deceased or alive), hospitalization periods, reason for hospitalization(s) and outcome should be confirmed by the Investigator via source data (eg, medical records) and recorded in the CRF.

All Study Periods, as applicable

Vital signs, physical examination, blood and urine samples and urine collection for efficacy and safety assessments, and 12-lead electrocardiogram (ECG) will be recorded as per the SOA. All endpoint-related laboratory assessments will be performed by a central laboratory.

Safety Reporting

All adverse events (AEs) will be collected from initiation of the first IMP administration through the Day 30 study visit. All serious adverse events (SAEs) will be collected from the signing of the informed consent form (ICF) through the end of study visit.

Biomarkers

Blood and urine samples will be collected and stored to enable exploratory analysis of kidney and cardiac biomarkers to explore potential changes between active treatment and placebo treatment that

may add to the understanding of the disease mechanism and how the treatment influences these mechanisms.

Blood samples will also be collected to enable analysis of immunologic biomarkers to explore the background of potential IRRs.

Randomization:

Randomization will occur in a 1:1 manner (RMC-035: placebo) and be stratified by region (North America and Europe) and screening eGFR (≥60 and <60 mL/min/1.73m^2) assessed at Day -1 (Visit 1), using the CKD-EPI equation with SCr.

Sample Size Justification:

For the primary endpoint, AKI 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%. A sample size of 268 subjects dosed leads to a test power of 80% to show statistically significant results at a two-sided significance level (alpha) of 0.10. An interim analysis (IA) of the primary endpoint will be performed when 50% of the planned subjects have completed Visit 6 (Day 5-9). Conditional power (CP) given 'the current trend' will be calculated. Sample size may be increased to a maximum of 348 subjects dosed. Sample size will not be decreased.

Number of Subjects (planned):

It is planned that approximately 380 subjects (approximately 30% screen failure assumed) will be enrolled to ensure 268 subjects dosed with either RMC-035 or placebo in a 1:1 ratio. Based on results at an interim analysis, up to 348 subjects may be dosed, requiring approximately 500 subjects to be enrolled.

Diagnosis and Main Criteria for Inclusion

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/1.73m^2 (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 m^2 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 treatment period, and for 7, 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 (see Appendix 1) starting at screening and throughout the treatment period, for 7 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 starting at screening, throughout the treatment period and for 7 days after the final IMP administration
8. Female subject must not donate ova starting at screening, throughout the treatment period and for 7 days after the final IMP administration
9. Male subject and their female spouse/partner(s) who are of childbearing potential must be using a condom starting at screening and continue to do so throughout the treatment period and for 7 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.
10. Male subjects must not donate sperm starting from screening, throughout the treatment period for up to 7 days 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

Waivers to the inclusion criteria will NOT be allowed.

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 severe allergic asthma defined as confirmed diagnosis of asthma poorly controlled while receiving high-dose inhaled corticosteroid treatment, or with requirement of a high level of treatment to maintain control
15. Subject has an ongoing chemotherapy or radiation therapy for malignancy that may have an impact on kidney function as assessed by the medical monitor
16. Subject has received an investigational medicinal product within the last 90 days (or within 5 half-lives of the investigational drug, whichever is longer)
17. Subject has a known allergy to RMC-035 or one of its constituents, or has previously received RMC-035

Investigational Product, Dosage and Mode of Administration:

RMC-035 (6.0 mg/mL Concentrate for Solution for Infusion) or matching placebo. Dosing will be based on renal function at Day -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
- Subjects with eGFR >30 and <60 mL/min/1.73m^2 will receive 0.65 mg/kg (per dose) for all five doses

The first (0h) and second (6h) doses will be administered as IV infusions over 60 minutes, and the third (12h), fourth (24h), and fifth (48h) doses will be administered as IV infusions over 30 minutes.

Duration of treatment:

Three days

Formal Stopping Rules for Investigational Medicinal Product Administration

Investigational Medicinal Product will be permanently discontinued in subjects who develop AKI stage 2 or higher (KDIGO guidelines) and in subjects meeting other criteria for IMP discontinuation per Section 5.3.1.

Concomitant Medication Restrictions or Requirements:

- Nephrotoxins (eg, non-steroidal anti-inflammatory drugs [NSAIDs]) should be discontinued before randomization and should not be used during the first 72 hours after first dose of IMP, unless strictly clinically indicated
- Angiotensin-converting enzyme inhibitors (ACEi)/angiotensin receptor blockers (ARBs) should be discontinued before randomization and should not be used during the first 72 hours after first dose of IMP, unless clinically indicated

Statistical Methods

Efficacy:

Efficacy analyses will be performed in the modified Intention to Treat (mITT) Analysis Set and will consist of all subjects in the Intention to Treat (ITT) set who received at least 1 dose of IMP.

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 (North America and Europe) and eGFR at Day -1 (≥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.

Secondary continuous efficacy endpoints, that are measures of renal function will be analyzed using robust regression of log-transformed values having sequentially multiply imputed any missing data. 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 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 AUC will be calculated using the trapezoidal rule with the actual times the measurements are taken. The relative difference (RMC-035 vs. placebo) and its 90% confidence interval will also be reported. Secondary binary efficacy endpoints will be analyzed using the same approach as specified above for the primary endpoint.

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 at baseline.

Exploratory Biomarkers:

--Plasma/serum, and urine samples will be collected and stored for potential future analysis of exploratory biomarkers, eg, urine (KIM-1, NGAL, TIMP2, IGFBP7, CCL-14, IL-18, LFABP); and

plasma/serum (NTproBNP, cTnI and/or cTnT). Additional urine and/or plasma/serum biomarkers related to the mode of action of RMC-035 (eg, endogenous A1M) may also be analyzed.

Immunologic Biomarkers:

Blood samples will also be collected to enable analysis of immunologic biomarkers exploring the background and mechanism of potential IRRs.

Safety:

Safety analyses will be conducted on the Safety Analysis Set (SAF), which includes all randomized subjects who received at least 1 dose of RMC-035. 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 dose group.

Interim Analysis:

An interim analysis (IA) of the primary endpoint will be performed when 50% of the planned randomized subjects have completed Visit 6 (Day 5-9). Conditional power (CP), the probability that the final analysis will be statistically significant, will be calculated. Sample size may be increased to a maximum of 348 randomized subjects. Sample size will not be decreased. The study may be stopped at the IA for futility reasons. Study eligibility criteria may also be modified.

Given the unblinded nature of this IA, an unblinded Data Monitoring Committee (DMC) of independent individuals not involved in any other study activities will be utilized. Further details on the IA and the control of the type I error will be provided in the DMC Charter and Statistical Analysis Plan.

An IA of exploratory urinary and blood biomarkers to further elucidate the mechanism of action of RMC-035, understand potential immunologic responses, and guide further development in additional indications may also be performed during the study at the discretion of the Sponsor.

Table 3: Schedule of Assessments – Complete Study Period

Assessments	**Screening**	**Treatment**								**Follow-Up**			
Visit Number	**1** [h]	**2** Day of Surgery						**3**	**4** (EOT)	**5** [v]	**6** (discharge)[w]	**7** [x]	**8** (EoS) [x, y]
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** [z]	**12h** [z]						
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	x
Serum Cystatin C [f]	x	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			

Assessments	**Screening**	**Treatment**								**Follow-Up**			
Visit Number	**1** [h]	**2** Day of Surgery						**3**	**4** (EOT)	**5** [v]	**6** (discharge)[w]	**7** [x]	**8** (EoS) [x, y]
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** [z]	**12h** [z]						
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			
Plasma sampling for immunologic biomarkers [s]		x						x, x [s]	x, x [s]				
HRQoL Assessments [t]	x												x
ADA assessment		x										x	x
Concomitant medication recording [u]	←	—	—	—	—	—	—	—	—	—	—	—	→
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 (Appendix 3) or per criteria in Section 5.3.1.

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

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. Plasma samples for assessment of immunologic biomarkers will be collected at the intervals described below, respectively:

Immunology Biomarker Sampling Study Day	Time Window	**Predose** ≤30 min	**90 min** ±20 min
Day 1	Start of Infusion 1 (t=0 h)	x	
Day 1	Start of Infusion 2 (t=6 h)		
Day 1	Start of Infusion 3 (t=12 h)		
Day 2	Start of Infusion 4 (t=24 h)	x	x
Day 3	Start of Infusion 5 (t=48 h)	x	x

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

u. 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.

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

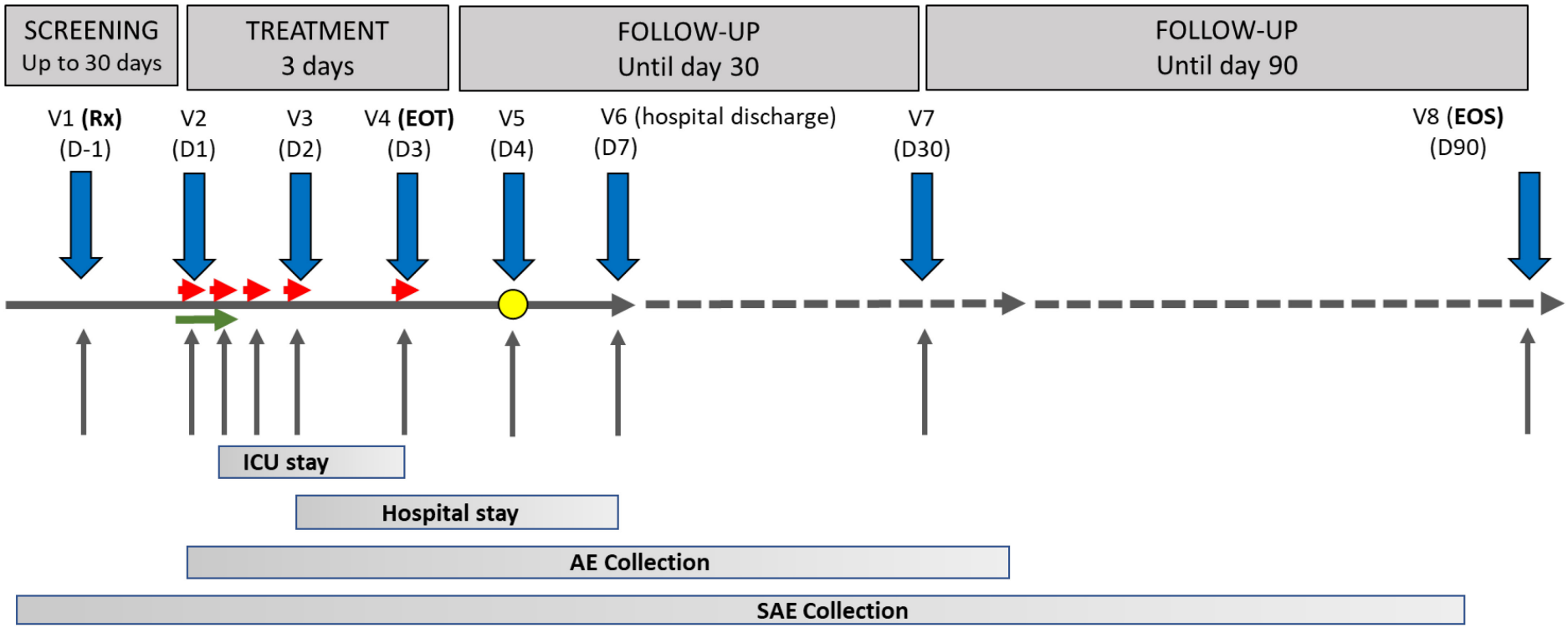
w. Visit 6 and all associated assessments should occur on the day of hospital discharge. In case subject is discharged on Day 4, discharge (Visit 6) assessments performed prior to discharge on that day are acceptable.

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

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

z. Assessments must be performed prior to IMP administration

Figure 1: Study Flow Chart

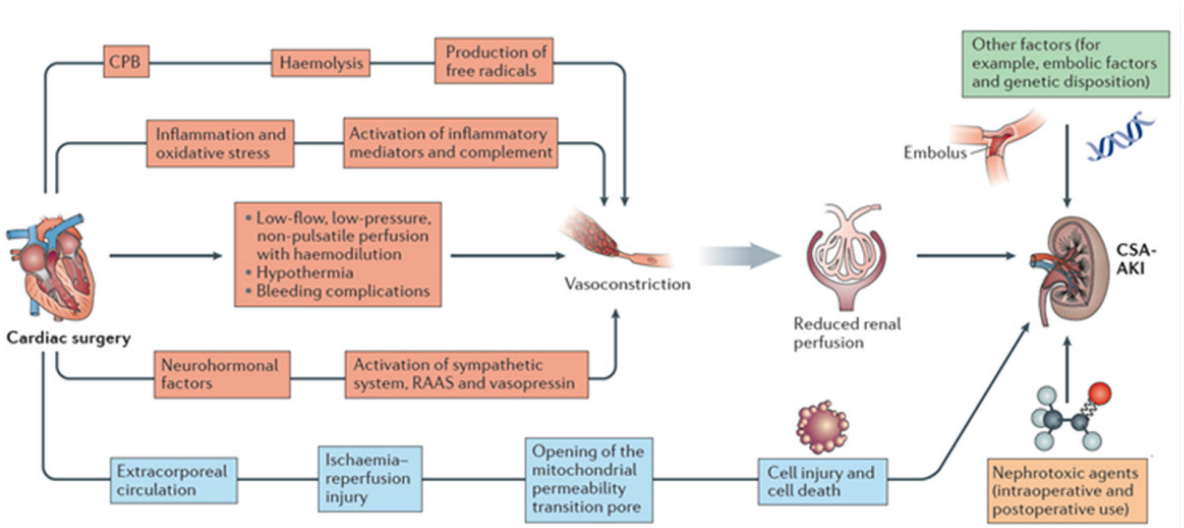


Rx = randomization
EOT = end of treatment
EOS = end of study
Study visit
PK and/or laboratory assessments
RMC-035 administration
Cardiac Surgery
Primary Endpoint Evaluation

2. INTRODUCTION

Acute Kidney Injury (AKI) is a clinical syndrome characterized by rapid loss of renal function and is associated with increased morbidity and short and long-term mortality. The pathophysiology of AKI is multifactorial and includes renal ischemia, heme toxicity, inflammatory responses, oxidative stress, and cytokine release amongst others (Figure 2).

Figure 2: Pathophysiological Pathways in CS-AKI



Cardiac surgery-associated acute kidney injury (CS-AKI) can be caused by reduced renal perfusion that results from vasoconstriction after cardiac surgery.
A number of pathophysiological pathways can lead to vasoconstriction (red boxes).
CS-AKI can also be caused by ischemia-reperfusion injury that occurs in extracorporeal circulation (blue boxes), leading to the opening of mitochondrial permeability transition pores in the kidneys and to cell injury or cell death.
In addition, nephrotoxic agents (orange box) and other factors (green box) can contribute to CS-AKI. CPB, cardiopulmonary bypass; RAAS renin-angiotensin-aldosterone system.
Source: (Wang 2017)

Acute kidney injury is a global public health concern impacting more than 13 million patients annually. It is associated with high morbidity and mortality, approximately 1.7 million deaths per year, and remains a major unmet medical need. AKI leads to long-term complications including the development of Chronic Kidney Disease (CKD), exacerbation of pre-existing CKD, and accelerating the progression to end-stage renal disease . Thus, a single AKI episode can lead to a significant burden of comorbidities, poor quality of life and high long-term costs .

AKI is frequently observed during hospitalizations and is considered one of the most important complications among hospitalized patients. A systematic review (2004 to 2012) of 154 large cohort studies was conducted to estimate AKI and its stages of severity and associated mortality. AKI definitions were reclassified according to the Kidney Disease Improving Global Outcomes (KDIGO 2012) staging system. The study included a total of more than 3.5 million patients and showed a pooled incidence rate of AKI of 21.6% in adults and 33.7% in children (Susantitaphong 2013).

AKI is a broad clinical syndrome encompassing various etiologies, including specific kidney diseases (eg, acute interstitial nephritis, acute glomerular and vasculitic renal diseases); non-specific conditions (eg ischemia, toxic injury); as well as extrarenal pathology (eg, prerenal azotemia, and acute postrenal obstructive nephropathy). More than one of these conditions may coexist in the same patient and, more importantly, epidemiological evidence supports the notion that even mild, reversible AKI has important clinical consequences, including increased risk of death (Section 2: AKI Definition (KDIGO 2012)).

Globally, an estimated 2 million cardiovascular surgeries (including coronary artery bypass grafting [CABG], Heart Valve operations and other) per year are performed (Parikh 2011). In 2014 in the USA there were 371,000 CABG procedures (Salim et al, 2020). According to data from the Organisation for Economic Cooperation and Development, CABG is on average performed at a rate of 44 per 100,000 individuals. The incidence of CABG was 79/100,000 in 2010 in the USA and with considerable differences between EU countries (91/100,000 in Hungary and 18/100,000 in Spain) (Head 2017). AKI is considered an important determinant of mortality in patients undergoing cardiovascular surgery (Lenihan 2013, Khadzhynov 2019).

The reported incidence of AKI in this setting varies depending on the specific population studied and the definitions used. The following publications are all on the basis of modern standardized definitions of AKI. Cardiac surgery related AKI (CS-AKI) occurs in up to 30% of cardiac surgery patients (Vives 2019). The pooled rate of cardiopulmonary bypass (CPB)-associated AKI was 18.2%, and of renal replacement therapy 2.1% (Pickering 2015). Approximately 18% of patients undergoing cardiac surgery experience AKI, and approximately 2-6% will require hemodialysis (Thiele 2015). In a global meta-analysis of 91 observational studies including more than 320,000 patients undergoing cardiac surgery, the pooled incidence of AKI was 22.3% (Stage 1 AKI: 13.6%, Stage 2 AKI: 3.8% and Stage 3 AKI: 2.7% and 2.3% of patients received renal replacement therapy). The pooled short-term and long-term mortality rate were 10.7% and 30% respectively and increased along with severity of stages (Hu 2016). 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 (Tinica 2020).

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 (McCullough 2016) and 71.7% in patients with a positive biomarker test (that identifies patients at very high risk for AKI) shortly after surgery (Meersch 2017). In a recent study of CS-AKI, in a population similar to that of 21-ROS-05 study, the pooled incidence of AKI was 34.2% (Serraino 2021) and in another recent study 28% patients developed CS-AKI (20%, 5% and 3% in KDIGO 1, 2 and 3, respectively) (Leballo 2021).

Current treatment strategies and clinical management of CS-AKI are mainly supportive, including optimal fluid management and maintenance of hemodynamic stability (O'Neal 2016). At present, no specific therapeutic interventions are available for prevention or treatment of CS-AKI. This is an area of large unmet medical need, where patients who develop post-surgery AKI are at risk for additional severe complications, including need for renal replacement therapy (RRT), progression to 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.

The investigational medicinal product (IMP), RMC-035, contains the pharmacologically active protein RMC-035, which is a recombinant variant of endogenous human alpha-1-microglobulin (A1M). Oxidative stress is a mechanism inherent to many disorders of acute cellular injury. The kidneys are particularly vulnerable to oxidative stress, hence the clinical development of RMC-035 is primarily focused on the prevention of AKI. RMC-035 has a short half-life and is mostly distributed to the kidney, and thus is well suited to treat acute renal conditions

Employing several antioxidation mechanisms, A1M can protect cells and tissues from various forms of cell damage caused by oxidative stress, including free radicals and reactive oxygen species (ROS) (Olsson 2008, Olsson 2010, May 2011, Olsson 2011). Four different molecular mechanisms have been implicated in the actions of A1M including heme binding, reductase activity, radical scavenging, and binding to, thereby protecting, mitochondria (Akerstrom 2014).

RMC-035 is being developed for the prevention of AKI in patients undergoing cardiac surgery and has been evaluated in four Phase 1 clinical studies: 17-ROS-01 (single ascending dose [SAD] study); 19-ROS-02 (multiple ascending dose [MAD] study); 19-ROS-03 (renal impairment study); and 20-ROS-04 (Phase 1b study in subjects undergoing nonemergent on-pump CABG and/or valve surgery. Based on available clinical and non-clinical data, RMC-035 is assessed as safe and generally well tolerated across study populations, ie in healthy subjects, renally impaired subjects and in cardiac surgery subjects, supporting its continued development in CS-AKI. This is considered a suitable indication for RMC-035 since it effectively targets common pathophysiological pathways, combined with a well-defined time point after the potential initial renal insult (ie at onset of CBP) as reference point for initiation of treatment.

Study Rationale

This is 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 AKI following open-chest cardiac surgery. This is the second RMC-035 clinical study in cardiac surgery subjects and is being conducted to provide proof-of-concept efficacy data and to guide further clinical development in this patient population. Based on an integrated assessment of available non-clinical and clinical data, the number of doses and infusion time are considered to be well-tolerated (see Section 6.2.1) and to provide targeted protection against oxidative stress and heme toxicity in this patient population, with the aim to prevent or ameliorate post-surgery renal injury.

2.1. Background

For a complete summary of the non-clinical and clinical RMC-035 development program, please refer to the Investigator's Brochure (IB).

2.1.1. Pharmacology

RMC-035 is a recombinant variant of the endogenous human protein A1M. RMC-035 is a potent antioxidant, harboring multiple physiological functions including reductase activity and radical scavenging, heme binding and protection of mitochondria during cellular stress. The key non-clinical data supporting the pharmacological utility of RMC-035 derives from animal models of ischemic AKI. In addition, a number of other animal models related to renal injuries, eg, due to heme or radiation exposure, has provided further support for the use of RMC-035 as a renal protector. Although experimentally diverse, the targeted pathophysiological mechanism in these

models is to a certain extent similar, where oxidative stress is considered to be a central disease mechanism.

2.1.2. Summary of Toxicology Studies

Good laboratory practice (GLP) repeated dose toxicity studies of intravenous (IV) administered RMC-035 have been conducted in rat (14 and 28 days of repeated dosing), marmoset (28 days repeated dosing) and cynomolgus monkey (3 days repeated dosing). In addition, exploratory non-GLP single and multiple dose toxicity studies have been conducted in rat, marmoset and cynomolgus monkey.

In marmoset and cynomolgus monkey GLP-studies, RMC-035 related histopathological changes in kidneys with tubular degenerative changes and casts were observed. Furthermore, in the cynomolgus monkey study, a transient increase in plasma creatinine and urea was observed. All changes appeared dose-dependent and were reversible after 4 weeks of recovery. Immuno-histochemical staining confirmed RMC-035 presence in casts, suggesting that pathological changes were the result of excessive protein filtration by the tubules at high RMC-035 doses leading to renal protein overload. In rat studies, no specific test-item related pathophysiological changes were recorded, and treatment related effects were limited to transient clinical signs (discomfort and piloerection).

The no observed adverse effect levels (NOAEL) were established in all GLP-studies and have been set at 20 (females) to 25 (males) mg/kg/day in rats, 10 mg/kg/day in marmoset and 10 mg/kg/day in the cynomolgus monkey. Target organ toxicity considered for NOAEL and dose setting is kidney tubular injury due to protein overload, the result of a high C_{max} (protein concentration) rather than cumulative exposure (area under the curve, AUC).

In more recent GLP studies, 14-day repeated dosing in rat and 3-day repeated dosing in cynomolgus monkey, provided NOAELs at higher exposure levels (269 and 161 µg/mL respectively). In rat repeat dose GLP studies, C_{max} exposures at NOAEL were observed up to 269 and 405 µg/mL (M&F) on day 1. However, as a conservative approach to dose setting, the marmoset 28-day repeat dose study C_{max} at 10 mg/kg NOAEL (60 µg/mL) has been employed for human dose setting and designation of exposure margins.

2.1.3. Summary of Completed Clinical Studies

2.1.3.1. Single Ascending Dose Study (17-ROS-01)

In study 17-ROS-01 (SAD), five dose levels were evaluated in healthy subjects in the dose range of 0.08 to 2.6 mg/kg. Briefly, six subjects were included in the first three cohorts (dose levels: 0.08, 0.24 and 0.72 mg/kg) to receive either RMC-035 or placebo (4:2 randomization) as a single IV infusion over 30 minutes. In cohort 4 and 5 (dose levels: 1.3 and 2.6 mg/kg), 8 subjects (6 on active and 2 on placebo) were included.

RMC-035 administered as single ascending doses (0.08 to 2.6 mg/kg) to healthy subjects was safe and well tolerated as assessed by adverse events (AEs), vital signs, electrocardiograms (ECGs), physical examinations and safety laboratory parameters. There were no deaths or other serious adverse events (SAEs) or AEs leading to discontinuation reported as related to RMC-035 during the study. One SAE of syncope following standing up from the bed was reported by one subject dosed with RMC035 at 2.6 mg/kg, which was not considered as related to IMP in a subject known to have a history of syncope.

There were no clinically significant abnormalities in any of the laboratory, vital signs or ECG, except a transient increase of white blood cell count at 12 hours after dosing in the highest dose group.

2.1.3.2. Multiple Ascending Dose Study (19-ROS-02)

In study 19-ROS-02 (MAD), three dose levels were evaluated: 0.43, 0.86 and 1.3 mg/kg per dose. Six healthy subjects were included per dose group to receive either RMC-035 or placebo (4:2 randomization) as multiple doses over 48 hours. The first dose was administered as an IV infusion over 60 minutes (infusion start at t = 0). Subsequent doses were administered at t = 6, 12, 24, 36 and 48 hours as IV infusions over 30 minutes.

RMC-035 was considered safe and well tolerated as assessed by AEs, vital signs, physical examinations, and safety laboratory parameters. There were no deaths or SAEs reported in this study. The most frequently reported event was local Infusion Site Reactions (ISRs) reported by the majority of subjects. Infusion Site Reactions were characterized by varying symptomatology including localized redness, tenderness, pain, and swelling. In one subject, an ISR led to early discontinuation after the fifth administration of IMP. Symptoms were generally mild in severity (only two subjects had ISRs of moderate severity) and lasted mostly for several days to 3 weeks in individual subjects.

Other events reported more frequently than in the placebo group were nausea and vomiting. All events were mild to moderate in severity and resolved spontaneously. There were no clinically significant changes reported in clinical laboratory, vital signs, and ECG assessments. A transient increase in leukocyte count was observed in higher dose groups at the 12 h time point, which was considered not clinically significant by the investigator. Furthermore, apparent reductions in serum creatinine, blood urea nitrogen, and uric acid (mainly in the 1.3 mg/kg group) were observed during the treatment period and at follow-up visits, which were all considered not clinically significant by the investigator.

2.1.3.3. Renal Impairment Study (19-ROS-03)

Study 19-ROS-03 is an open-label Phase 1 study to investigate the effect of renal function on the pharmacokinetics, safety and tolerability of RMC-035.

In total, eight subjects with estimated glomerular filtration rate (eGFR) ≥15 and <90 mL/min/1.73 m^2 not on dialysis were included and allocated into one of five eGFR strata based on renal function at screening. Distribution of subjects were as follows: 1 subject in stratum 1 (≥75-<90 mL/min/1.73 m^2), 1 subject in stratum 2 (≥60-<75 mL/min/1.73 m^2) and 2 subjects per stratum in strata 3 to 5, ie, ≥45-<60 mL/min/1.73 m^2, ≥30-<45 mL/min/1.73 m^2 and ≥15-<30 mL/min/1.73 m^2, respectively. The screening eGFR of included subjects ranged from 16 to 75 mL/min/1.73 m^2 (Modification of Diet in Renal Disease equation).

All subjects received a single IV infusion of RMC-035 over 30 minutes. Dose levels were set based on available interim pharmacokinetic (PK) data in the MAD study: subjects in strata 1 to 4 received 0.43 mg/kg and subjects in stratum 5 received 0.22 mg/kg.

RMC-035 was considered safe and well tolerated based on AEs, vital signs, ECGs, physical examinations and safety laboratory parameters. There were no deaths or other SAEs or AEs leading to discontinuation during the study. There were no clinically significant abnormalities in any of the laboratory, vital signs or ECGs. Only one AE was reported in one subject (reported

term feeling cold). The event was of mild severity, possibly related to IMP and resolved spontaneously approximately 2 hours after onset.

The plasma clearance of RMC-035 correlated to renal function across the spectrum of eGFR and declined with lower renal function. AUC was increased in subjects with renal impairment as compared to healthy subjects, whereas the C_{max} remained essentially unaltered.

2.1.3.4. Exploratory Safety Study in Cardiac Surgery Subjects (20-ROS-04)

Study 20-ROS-04 is a Phase 1b, randomized, double-blind, parallel treatment group study to evaluate the safety, tolerability and pharmacokinetics of RMC-035 in subjects undergoing non-emergent on-pump Coronary Artery Bypass Graft (CABG) and/or valve surgery.

This was the first clinical study in cardiac surgery subjects and was conducted to guide further clinical development in this patient population. Primary endpoint was the nature, frequency and severity of AEs. Twelve subjects undergoing elective on-pump CABG and/or valve surgery with additional risk factors to develop CS-AKI were enrolled in the study; 8 subjects received RMC-035 and 4 received placebo (2:1 randomization).

The study consisted of three study periods: Screening (Day -1), Treatment period (Day 1-3) and a Safety follow-up period (Day 4-31). A total of five doses of RMC-035 or placebo were administered as IV infusions during the treatment period. First dose of RMC-035 was 1.3 mg/kg or placebo and was administered over 60 minutes. Dose administration started during surgery, approximately 10 minutes before the expected start of CPB. Second, third, fourth and fifth doses of RMC-035 were 1.3 mg/kg per dose or placebo and were administered over 30 minutes at 6, 12, 24, and 48 hours after the start of the first dose administration. The dose of RMC-035 was reduced by 50% (ie 0.65 mg/kg per dose administration) in subjects with eGFR 30 to <60 mL/min/1.73 m^2 at screening. After the first 2 doses, further permanent dose reductions were mandated (guided by Creatinine Clearance and/or eGFR) to either 0.65 mg/kg or 0.43 mg/kg if Creatinine Clearance and/or eGFR declined below 60 or 30 mL/min, respectively.

Preliminary review of the safety results support that RMC-035 was generally well tolerated based on AEs, vital signs, ECGs, physical examination and safety laboratory parameters. AEs and SAEs were reported mainly during and just after surgery and were within the expected range of events in relation to the surgical procedure. There were no reports of treatment related AEs. The only events reported in more than one subject were acute kidney injury, pleural effusion and pericardial effusion. No ISRs were reported and no ADAs were detected in this study.

One subject receiving RMC-035 died at Day 12 due to surgical complications: a rupture of a coronary vessel with cardiac tamponade followed by subsequent persistent hypoperfusion, cardiogenic shock and initiation of dialysis treatment on Day 2. The subject later developed sepsis due to a caecal perforation which was assessed to be caused by a non-occlusive mesenterial ischemia on the basis of long-term hypoperfusion with inotropic support.

One subject receiving RMC-035 started with dialysis at Day 3. The subject underwent a complex CABG surgery (CPB x 2, total surgical time above 6 hours) for the third time and had a low renal function prior to surgery (pre-operative eGFR 30 mL/min/1.73 m^2) with a history of prolonged dialysis treatment after the previous cardiac surgery.

For further details, please see the Investigator's Brochure.

2.1.4. Key Safety Information

Preclinical experience includes an extensive safety toxicology program with GLP studies with up to 28 days once daily IV dosing in rats and marmosets as well as once daily dosing for three days in cynomolgus monkeys.

The only identified target organ is the kidneys; the principal findings were renal tubular histopathology and associated changes to renal function biomarkers related to higher exposures and short infusion times that resulted in high plasma RMC-035 concentrations (C_{max}) and subsequent tubular protein (RMC-035) overload. Nonclinical studies support that renal findings are primarily related to the peak concentration (C_{max}) and not cumulative exposure (AUC) of RMC-035. Importantly, no adverse renal effects of RMC-035 were observed in clinical studies.

The maximum C_{max} levels in the SAD study was 38.8 µg/mL (2.6 mg/kg) and in the MAD study 16.3 µg/mL (when administered 6 times over 48 hours at 1.3 mg/kg). In cardiac surgery subjects (Study 20-ROS-04) the maximum observed C_{max} was 23.3 µg/mL following a 30-minute infusion (1.3 mg/kg) and 13.1 µg/mL following a 60-minute infusion at the same dose level. These levels are still below the NOAEL levels of 60 µg/mL (marmoset 28-day study) and 161 µg/mL (cynomolgus 3 day study) in the relevant toxicology studies.

RMC-035 was so far evaluated in 52 subjects in four clinical studies including healthy subjects (17-ROS-01 and 19-ROS-02), renally impaired subjects across a wide spectrum of renal function (19-ROS-03) and in cardiac surgery subjects (20-ROS-04), respectively. RMC-035 was generally well tolerated, and the most commonly reported adverse events (mostly mild) in healthy subjects receiving peripheral dose administrations were non-serious local infusion site reactions (ISRs), headache and gastro-intestinal events (abdominal discomfort, nausea and vomiting). Notably, no ISRs were reported in single dose studies (SAD and Renal Impairment), and more importantly, in cardiac surgery subjects receiving five dose administrations of RMC-035 via a central venous catheter at relatively higher infusion flow rates.

In Study 20-ROS-04 including subjects undergoing open-chest cardiac surgery with additional AKI risk factors (8 received RMC-035, 4 received placebo), the only AEs reported in more than one subject were acute kidney injury, pleural effusion and pericardial effusion. These events, and other AEs in the study which were single events, reflect the type of events that would be anticipated in the underlying patient population undergoing cardiac surgery. No anti-drug antibodies (ADAs) were detected in cardiac surgery subjects receiving multiple dose administrations via central venous catheters.

Overall, RMC-035 is generally well tolerated across study populations and supports its further evaluation in a larger multi-centre phase 2 study.

Update from an early blinded review of the present study:

For a detailed overview of the key safety information, please see the Investigator's Brochure. A blinded review of safety data from the ongoing study 21-ROS-05 showed that a relevant number of study subjects had experienced single or repeat episodes of non-serious chills or potential infusion related reactions. In some cases, the chills were associated with pyrexia, hypertension, and/or tachycardia. Onset of events was generally within 30 to 60 minutes and most common following completion of the 4th and/or 5th infusion. More details can be found in the Investigator's Brochure.

2.1.4.1. Risk Mitigation Measures

ISRs

The only identified risk of RMC-035 (ie observed in clinical studies) is non-serious local infusion site reactions (ISRs) which were reported in the majority of healthy subjects receiving multiple peripheral dose administrations. No ISRs were reported in single dose studies of RMC-035 or in cardiac surgery subjects who received multiple dose administrations in a central venous line. Overall, the risk for ISRs is therefore considered to be low, with the following risk mitigation measures being applied for this study:

- Administration of IMP in a central venous line whenever possible (use of central venous catheters is considered standard of care in cardiac surgery)
- Dilution of IMP in a sodium chloride infusion buffer prior to administration and administration rate with higher infusion rate to minimize local irritancy
- Discontinuation of IMP in case a subject develops an ISR grade 3 or higher and which is considered immune related

The infusion site will be carefully monitored, however should local irritancy still arise, it should be managed by standard of care treatment when clinically indicated, eg, with topical anti-coagulants and cooling

Chills and infusion related reactions (IRR)

A blinded review of safety data from the ongoing study 21-ROS-05 showed that a relevant number of study subjects had experienced single or repeat episodes of non-serious chills or other signs suggestive of IRR.

The following risk management measures apply:

- Exclusion of subjects with severe asthma
- Close observation of patients during and after the administration of the IMP infusions, in particular after the 4th and 5th dose
- Management of signs or symptoms suggestive of IRR according to local standard of care including a recommendation for pre-treatment with paracetamol (acetaminophen)
- Discontinuation of IMP in case a subject develops an IRR grade 3 or higher and which is considered immune related

Renal tubular pathology (renal protein overload)

Renal protein overload was observed at high peak concentrations of RMC-035 in nonclinical toxicology studies and remains a potential risk. No evidence for tubular cell stress/damage or other adverse renal effects of RMC-035 were observed in clinical studies. The following risk mitigation measures are applied:

- The maximum clinical dose is 1.3 mg/kg per dose administration. This dose has already been proven safe and well tolerated in healthy subjects (Study 17-ROS-01 and 19-ROS-02) and in cardiac surgery subjects (Study 20-ROS-04)

- Reduced dose to 0.65 mg/kg for subjects with eGFR<60 mL/min/1.73m2 as modeling of pharmacokinetic (PK) data in renally impaired subjects demonstrated an approximate doubling of RMC-035 exposure (AUC) at this eGFR level
- Exclusion of subjects with baseline eGFR<30 mL/min/1.73 m2
- Permanent discontinuation of IMP if development of AKI stage 2 or higher as per KDIGO criteria (also including renal replacement therapy, ie, dialysis)
- Monitoring of renal function parameters (serum creatinine [SCr], cystatin C, eGFR) will be performed throughout the study

Development of ADAs

Confirmatory ADAs were observed in a few subjects receiving a single dose administration of RMC-035 (Study 17-ROS-01) and in the majority of subjects receiving multiple dose administrations of RMC-035 (Study 19-ROS-02) with peripheral dose administration. No ADAs were detected in cardiac surgery subjects receiving central venous dose administrations (Study 20-ROS-04) and no adverse consequences of ADA formation have been observed in clinical trials of RMC-035.

Potential risks associated with ADAs are considered limited given the short treatment duration (<3 days) and rapid initial plasma clearance of RMC-035. Accordingly, ADAs are not expected to impact the PK or pharmacological properties of RMC-035. The risk for unforeseen cross-reactions due to re-exposure of RMC-035 is mitigated by exclusion of subjects who were previously exposed to RMC-035. Furthermore, any potential cross-reaction with endogenous A1M is unlikely to impact the native protein function due to its high abundance in plasma and high synthesis and turnover rate (Gunnarsson 2017). Analysis of ADAs from Study 19-ROS-02 also demonstrates lack of cross-reactivity with endogenous A1M.

Potential ADAs in this study will be measured and further characterized, eg regarding Ig subtype, neutralizing capacity and cross-reactivity, to support and guide further clinical development of RMC-035.

2.1.5. Risk / Benefit Assessment

More detailed information about the known and expected benefits and risks and expected adverse events of RMC-035 may be found in the IB.

Efficacy of RMC-035 for prevention of AKI is currently unknown. Based on the experimental data from relevant animal studies and the mechanism of action of RMC-035 towards relevant pathophysiological pathways of CS-AKI it is anticipated that RMC-035 will reduce the occurrence and severity of AKI in subjects undergoing cardiac surgery who are at high risk for development of AKI. Prevention of AKI is expected to provide clinical benefit for subjects undergoing cardiac surgery.

All subjects will receive the current standard of care. For subjects allocated to receive placebo, no additional benefit can be expected.

RMC-035 has been evaluated in healthy subjects, subjects with reduced renal function and subjects undergoing cardiac surgery. No serious adverse reactions (SARs) have been identified with RMC-035 treatment. The identified risk of non-serious local ISRs and potential risks of renal protein overload and ADA formation, respectively, are considered limited and manageable

in the target patient population with a short treatment duration (<3 days). Appropriate risk mitigation measures (see Section 2.1.4.1) have been implemented in the design of this study.

Taking the hypothesized efficacy to prevent AKI and the potential risks identified in association with RMC-035 into account the risk-benefit is considered favorable, justified and well-balanced for subjects undergoing cardiac surgery who are at high risk to develop AKI.

3. TRIAL OBJECTIVES AND ENDPOINTS

3.1. Primary, Secondary, and Exploratory Objectives

3.1.1. Primary Objectives

- To evaluate the efficacy of RMC-035 for prevention of AKI (KDIGO definition) in subjects undergoing CABG and/or valve surgery and/or aorta surgery with additional risk factors for developing cardiac surgery associated AKI
- To evaluate the safety and tolerability of RMC-035

3.1.2. Secondary Objectives

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
    - 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)
- 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

3.1.3. Exploratory Objectives

- To evaluate post-baseline changes in kidney and cardiac biomarkers
- To evaluate changes in immunologic 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

3.2. Primary, Secondary, and Exploratory Endpoints

3.2.1. 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)

3.2.2. Primary Safety Endpoint

- Nature, frequency and severity of treatment-emergent adverse events (TEAEs)

3.2.3. 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

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)
- 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 C_{max})
- 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)

3.2.4. 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)

Changes in immunologic biomarkers

- Immunologic biomarkers to explore the background and mechanism of potential IRRs including but not limited to markers of complement activation, cytokine release and mast cell activation.

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)

4. INVESTIGATIONAL PLAN

4.1. Overall Study Design

This is a Phase 2, randomized, double-blind, adaptive, parallel group clinical study that will evaluate RMC-035 compared to placebo in approximately 268 subjects at high risk for AKI following open-chest cardiac surgery (based on predefined risk factors). An interim analysis, including a sample size re-assessment, will be performed, during which subject enrollment will continue. Another IA involving exploratory biomarkers may be performed at the Sponsor's discretion. The study consists of 8 visits for each subject with four study periods:

- Screening Period: 30 days (Day-30 to -1)
- Treatment Period: 3 days (Day 1-3)
- Follow-Up Period: 27 days (Day 4-30)
- Extended Follow-Up Period: 60 days (Day 31-90)

See the Schedule of Assessments (SOA, Table 3) for the allowed time window for each study visit. During the course of the study, visits and assessments will be performed as defined in the SOA.

Screening Period and Randomization (Visit 1)

Prospective subjects may be consented within 30 days (inclusive) prior to the date of scheduled surgery. Subjects who have signed the informed consent may undergo initial screening assessments during the screening period (full physical examination, medical history, clinical laboratory testing, completion of health-related quality of life questionnaires etc.) as described in the SOA (Table 3).

All screening clinical laboratory testing to confirm eligibility and determine starting dose based on renal function will be performed by local laboratory on Day -1 (Visit 1), ie, the day before surgery is intended. In addition, sampling for serum creatinine and cystatin C will be collected for central laboratory analysis and will provide the baseline values for the purpose of relevant endpoint assessment.

Eligible subjects who comply with all inclusion criteria and none of the exclusion criteria will be randomized on Day -1, Visit 1 to receive either RMC-035 or placebo in a 1:1 randomization ratio. Both region (North America and Europe) and Day -1 eGFR calculated using 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.

Note: Some or all screening visit assessments listed in the SOA, including randomization, may be performed prior to surgery on Day 1 (Visit 2), ie, the day of surgery, if feasible for the site. All screening labs and assessments performed on Day 1 must be collected prior to anesthesia.

Note: In case surgery is postponed after randomization has occurred, re-assessment of SCr and body weight for the calculation of eGFR is not required. However, if re-assessment is done as part of standard of care, and the results indicate an eGFR decrease that places the subject in the lower eGFR category (ie <60 mL/min/1.73m^2 for a subject who was randomized within the ≥60 mL/min/1.73m^2 stratum) a decrease of the IMP dose to that of the lower eGFR category is acceptable if the change is clinically significant in the opinion of the investigator.

Treatment Period (Visits 2-4)

Surgery will occur on Day 1 (Visit 2). Before administration of IMP, preoperative safety assessment and sampling for local and central clinical laboratory testing will be performed per the SOA. Centrally processed laboratory samples of morning urine collected pre-surgery on Day 1 will provide the baseline values used for the purpose of relevant endpoint evaluations (UACR and UPCR).

The first administration of IMP will be given during surgery, to be started approximately 10 minutes before the expected initiation of CPB, as an IV infusion. The start time of the first infusion is time 0, the reference point for further dose administrations and protocol-mandated assessments. In total, 5 doses of RMC-035 or matching placebo will be administered during the treatment period. Administration of subsequent IV infusions start at 6, 12, 24 and 48 hours, respectively, after the start of the first dose administration.

IMP will be administered per the schedule below:

- The first (0h) and second (6h) doses are administered as IV infusions over 60 minutes
- The third (12h), fourth (24h) and fifth (48h) doses are administered as IV infusions over 30 minutes

Differences between eGFR at Day -1 (randomization) and additional eGFR readings collected on Day 1 prior to surgery as part of the study or per the investigational site's standard of care will not affect eligibility and starting dose unless the change is a decrease that the investigator considers clinically significant and that implies the subjects should have a lower starting dose. Should the subject eGFR drop below 30 ml/min/1.73m2, and in the investigator's opinion the decrease is clinically significant, the subject is no longer fulfilling eligibility criteria and should not start treatment.

IMP should preferably be administered through a central venous catheter (CVC) in a lumen which is reserved for the IMP. If the CVC is removed during the treatment period, the IMP may be administered via a catheter in a peripheral vein. This catheter should preferably be reserved for IMP administration only.

Surgery assessments (specified in the SOA and Section 8.6.1) performed before, during, and after surgery will be collected per standard of care and documented in the case report form (CRF). All medications administered before and during surgery, including anesthesia, will be recorded as concomitant medications. Postoperative admission of the subject to the ICU will also be recorded in the CRF.

Urine will be collected starting on Day 1 prior to surgery and will continue only for as long as the subject has a catheter (Foley) in place to calculate urine output (UO), or until 72 hours after first dose of IMP (if catheter remains in place). While the subject remains hospitalized in the ICU, site staff will measure UO in hourly intervals or per site's standard of care. If the subject is transferred to a non-ICU ward, UO will be measured in 6-hour intervals (see Section 8.8). Urine output (volume) will be entered into the CRF at 6-hour time periods as much as possible. The exact time period for the collection and the collected volume will be recorded in the CRF.

If the Foley catheter is removed, urine production will no longer be entered in the CRF but spot urine samples will continue to be collected as per SOA. UO can only be used for diagnosing AKI or rating AKI severity if UO has been calculated based on UO measured by a Foley catheter. If the Foley catheter is removed, UO will not be used for the AKI definition.

In case the subject is discharged from the ICU to a non-ICU ward prior to last dose administration, the remaining dose(s) will be administered either by study-trained non-ICU staff, or preferably, by study-trained ICU staff at the non-ICU ward. Blood and spot urine samples will be collected every day during the treatment period as per SOA, ie, 24 hours after start of initial IMP infusion (Day 2, Visit 3) and 48 hours after start of initial IMP infusion (Day 3, Visit 4; End-of-Treatment [EOT]). At a minimum, all subjects will remain hospitalized for the first 72 hours post-surgery (until completion of Visit 5).

Follow Up Period (Visits 5-7)

After the final dose has been administered (Day 3, Visit 4, EOT), additional study visits will be conducted at 72 hours after first dose of IMP (Day 4, Visit 5, primary endpoint assessment) and between Day 5-9 (Visit 6, Discharge) where blood and spot urine samples will be taken as per SOA. When the subject is discharged from the ICU either to the hospital ward, another treatment facility or home, this will be recorded separately in the CRF.

Note: Visit 6 and all associated assessments and sample collections should occur on the day of discharge from hospital. If Visit 6 samples are collected on day of scheduled discharge, but subject discharge is subsequently delayed, these samples should still be processed and entered as Visit 6 (discharge). In case a subject is not scheduled to be discharged until after the Visit 6 scheduling window (Days 5-9), it is preferred that Visit 6 (discharge) samples be collected on Day 7. In case subject is discharged on Day 4, Visit 6 (discharge) samples collected prior to discharge on that day are acceptable.

Subjects will initially be followed up for one month after surgery until the next study visit (Day 30, Visit 7). This visit may be performed by qualified and trained study staff at the investigational site, at the subject's home, or other suitable location, where appropriate. Blood and urine samples collected will be processed centrally. During this follow-up period, all AEs need to be reported to the Investigator as soon as possible, eg, by phone. The Investigator will decide whether a visit to the clinic or hospital needs to be scheduled.

Extended Follow Up Period (Visit 8)

A final study visit will be conducted at 3 months (Day 90) after surgery (Visit 8, End of Study [EOS]). This visit may be performed by qualified and trained study staff at the investigational site, at the subject's home, or other suitable location, where appropriate. Health related quality of life questionnaires will be collected. Blood and urine samples collected will be processed centrally. Subjects will be requested to provide information about hospitalization periods and other events qualifying as SAEs. Vital status (ie, deceased or alive), hospitalization periods, reason for hospitalization(s) and outcome should be confirmed by the investigator via source data (eg, medical records) and recorded in the CRF.

Upon completion of all EOS visit procedures, subjects will have completed the study.

All Study Periods, as applicable

Vital signs, physical examination, blood and spot urine samples and urine collection for efficacy and safety assessments, 12-lead ECG, as well as samples for PK analysis, ADA and exploratory biomarkers will be collected and recorded as per the SOA.

4.2. Participating Sites

Approximately 30 centers located in North America and Europe will participate in this study. During the conduct of the study, additional regions, countries or sites may be added, as necessary.

4.3. Number of Subjects

It is planned that approximately 380 subjects (approximately 30% screen failure assumed) will be enrolled to ensure 268 subjects dosed with either RMC-035 or placebo in a 1:1 ratio. Based on results at an interim analysis, up to 348 subjects may be dosed, requiring approximately 500 subjects to be enrolled.

4.4. Replacement of Subjects

Subjects who are randomized but do not receive the first dose of IMP may be replaced; these subjects (randomized but not dosed) will be followed for safety (SAE) and for the following endpoints:

- Acute kidney injury within 72 hours after the first dose of IMP (Visit 5, Day 4)
- Time-corrected AUC of SCr for Day 1 to Day 4 (72 hours after first dose of IMP)
- Major adverse kidney event at Day 30 (Visit 7) and Day 90 (Visit 8, EOS)

Additional assessments are not required to be collected.

Subjects included to replace others will be randomized (ie, may not be automatically allocated to the same arm to the replaced subject).

Any subject receiving at minimum the first dose of IMP will not be replaced.

4.5. Completion of Study

Completion of the study is defined as the time at which the last randomized subject completes the final follow-up period visit, is considered lost to follow-up, withdraws consent, or dies.

4.6. Early Study Termination

The Sponsor reserves the right to close the investigational site or terminate the study at any time for any reason at the sole discretion of the Sponsor. The investigational site will be closed upon study completion.

In the case of early termination of the study, the Sponsor (or designee) will provide written notification to the Investigators and regulatory authorities specifying the reason(s) for termination. The Investigator will be responsible for notifying their institution's Institutional Review Board (IRB) / Independent Ethics Committee (IEC), if appropriate.

4.6.1. Discontinuation of a Site

The Investigator may initiate study-site closure at any time, provided there is reasonable cause and sufficient notice is given in advance of the intended termination.

Reasons for the early closure of a study site by the Sponsor or Investigator may include but are not limited to:

- Failure of the Investigator to comply with the protocol, the requirements of the IRB/IEC or local health authorities, the Sponsor's procedures, or Good Clinical Practice (GCP) guidelines
- Inadequate recruitment of subjects by the Investigator
- Determination of unexpected, significant, or unacceptable risk to subjects
- Insufficiently complete and/or evaluable data.
- Plans to modify, suspend, or discontinue the development of the IMP

A study site is considered closed when all required documents and study supplies have been collected and a study-site closure visit has been performed.

The Investigator will complete the study and approve the final CRFs in satisfactory compliance with the protocol.

5. SELECTION AND WITHDRAWAL OF SUBJECTS

5.1. Subject 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 m^2 (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/1.73m^2 (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 (see Appendix 3)
    - 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 m^2 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 treatment period, and for 7 days after the final 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 (see Appendix 1) starting at screening and throughout the treatment period, for 7 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 starting at screening, throughout the treatment period and for 7 days after the final IMP administration
8. Female subject must not donate ova starting at screening, throughout the treatment period and for 7 days after final IMP administration
9. Male subject and their female spouse/partner(s) who are of childbearing potential must be using a condom starting at screening and continue to do so throughout the treatment period and for 7 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.
10. Male subjects must not donate sperm starting from screening, throughout the treatment period for up to 7 days 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

Waivers to the inclusion criteria will NOT be allowed.

5.2. Subject 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, Appendix 3) 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 severe allergic asthma defined as confirmed diagnosis of asthma poorly controlled while receiving high-dose inhaled corticosteroid treatment, or with requirement of a high level of treatment to maintain control
15. Subject has an ongoing chemotherapy or radiation therapy for malignancy that may have an impact on kidney function as assessed by the medical monitor
16. Subject has received an investigational medicinal product within the last 90 days (or within 5 half-lives of an investigational drug, whichever is longer)
17. Subject has a known allergy to RMC-035 or one of its constituents, or has previously received RMC-035

5.3. Subject Withdrawal Criteria

A subject may withdraw from the study at any time at his/her own request or may be withdrawn at any time at the discretion of the Investigator for safety, behavioral, compliance, or administrative reasons.

If a subject withdraws consent or is withdrawn by the Investigator, the Investigator must ask the subject if he/she is willing, as soon as possible, to have all EOS assessments performed as described in the SOA. Any ongoing SAE at the time of withdrawal will be followed as described in Section 9.7.

Subject data collected up to withdrawal of consent will be retained and included in the analysis of the study, and where permitted by local regulations, publicly available data (death records) can be included after withdrawal of consent. If the subject withdraws consent for disclosure of

future information, the Sponsor may retain and continue to use any data collected before such a withdrawal of consent.

If a subject withdraws from the study, he/she may request destruction of any samples taken and not tested, and the Investigator must document this in the site study records.

Although a subject is not obliged to give his/her reason(s) for withdrawing, the Investigator must make a reasonable effort to ascertain the reason(s), while fully respecting the subject's rights. Where the reasons are obtained, the primary reason for withdrawal must be specified in the End-of-Treatment/study form in the CRF.

5.3.1. Discontinuation of Investigational Medicinal Product

5.3.1.1. 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 SOA until the EOS visit:

- Development of stage ≥2 AKI, according to KDIGO definition (see Appendix 3)
- Need for RRT, ie, dialysis, including continuous RRT
- Need for percutaneous or surgical mechanical circulatory support (MCS) or extra-corporeal membrane oxygenator (ECMO)
- Reporting of a grade 3 (per Table 8, Section 9.3) 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:

- ALT > 3 × ULN combined with total bilirubin > 2 × ULN in the same sample
- 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)
- 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
- 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. See Appendix 2 for more detailed instructions.

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

5.3.1.2. Temporary discontinuation IMP

A temporary discontinuation of IMP may be considered if, in the opinion of the investigator, a subject's medical condition precludes IMP dosing due to a documented serious safety concern.

In case of a temporary discontinuation of IMP, the missed dose will not be replaced or administered at a later time point.

5.3.2. Lost to Follow-Up

A subject will be considered lost to follow-up if he or she repeatedly fails to return for scheduled visits and is unable to be contacted by the study site.

The following actions must be taken if a subject fails to return to the clinic for a required study visit:

- The site must attempt to contact the subject and reschedule the missed visit as soon as possible, counsel the subject on the importance of maintaining the assigned visit schedule, and ascertain whether or not the subject wishes to and/or should continue in the study.
- Before a subject is deemed lost to follow up, the Investigator or designee must make every effort to regain contact with the subject (where possible, 3 telephone calls and, if necessary, a certified letter to the subject's last known mailing address or local equivalent methods). These contact attempts should be documented in the subject's medical record.
- Should the subject continue to be unreachable, he/she will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

6. TREATMENT OF SUBJECTS

6.1. Investigational Medicinal Product

The IMPs for this study are RMC-035 or matching placebo.

6.1.1. RMC-035

RMC-035 6.0 mg/mL Concentrate for Solution for Infusion (RMC-035) contains the pharmacologically active protein RMC-035, a recombinant variant of endogenous human A1M. Properties and appearance of RMC-035 are described in Table 4.

Table 4: RMC-035 Characteristics

Pharmaceutical form:	RMC-035 is a concentrate (6.0 mg/mL) for solution for infusion for IV administration.
Appearance:	Clear, colorless to slightly yellowish solution. Practically free from visible particles, may contain translucent-to-white proteinaceous particles
Excipients:	Tris 10 mM pH 8.0.
Packaging:	RMC-035 is filled in 10 mL glass vials, closed with a 20 mm rubber stopper and aluminum crimp with a plastic cap.
Storage:	RMC-035 vials should be stored at -20 ± 5ºC (-4 ± 10ºF) and can be kept at room temperature (not above +25ºC (77°F)) for maximum 6 hours. RMC-035 diluted in 0.9% sodium chloride solution can be stored for 72 hours at 2-8ºC (36-46°F), or a shorter time period as specified in the Pharmacy Manual, if applicable.

Abbreviations: IV=intravenous

6.1.2. Placebo

The formulation of the matching placebo is identical to RMC-035 except that it contains no active ingredient. Placebo vials should be stored at -20 ± 5°C (-4°F) and can be kept at room temperature (not above +25ºC) for maximum 6 hours. Placebo diluted in 0.9% sodium chloride solution can be stored for 72 hours at 2-8°C (36-46°F).

6.1.3. Concomitant Medications

Concomitant medication refers to all drugs and therapies used from the time the Informed Consent Form (ICF) is signed through the end of study participation, including all medications used during surgery (for instance anesthesia).

Any prescription and non-prescription medications (eg, over-the-counter drugs and herbal supplements) that the subject is receiving at the time of screening or receives during the study must be recorded along with:

- Trade name or generic name
- Indication/reason for use
- Dates of administration including start and stop dates
- Dosage information including dose, route, and frequency

Changes, additions, or discontinuations to medications will be assessed and recorded in the CRF during each study visit. If a change is due to an AE, then this must be reported according to Section 9.7. The Sponsor should be contacted if there are any questions regarding concomitant or prior therapy.

Treatment and prevention of potential IRRs (see section 2.1.4.1) should follow local standard of care. This includes a recommendation to administer paracetamol (acetaminophen) during the treatment period.

6.1.3.1. Restricted Concomitant Medications

The following medications are prohibited prior to randomization into the study:

- Nephrotoxins (eg, non-steroidal anti-inflammatory drugs [NSAIDs]) should be discontinued before randomization and should not be used during the first 72 hours after first dose of IMP, unless strictly clinically indicated
- Angiotensin-converting enzyme inhibitors (ACEi)/angiotensin receptor blockers (ARB) should be discontinued before surgery and should not be used during the first 72 hours after first dose of IMP, unless clinically indicated

6.2. Investigational Medicinal Product Administration

Dose levels of IMP will be predefined based on renal function (eGFR calculated using the CKD-EPI equation with SCr) at screening (Day -1):

- Subjects with eGFR ≥60 mL/min/1.73m2 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
- Subjects with eGFR >30 and <60 mL/min/1.73m^2 will receive 0.65 mg/kg (per dose) for all 5 doses

The start time of the first infusion of IMP is time 0, the reference point for further dose administrations. In total, 5 doses of IMP or matching placebo will be administered during the treatment period. Administration of subsequent IV infusions will start at 6, 12, 24 and 48 hours, respectively, after the start of the first dose administration.

The first (0h) and second (6h) dose are administered as IV infusions over 60 minutes. The third (12h), fourth (24h) and fifth (48h) doses are administered as IV infusions over 30 minutes.

Guidelines for storage, preparation and administration of the IMP are outlined in Section 7; for detailed administration instructions, refer to the Pharmacy Manual.

6.2.1. Rationale for RMC-035 Dosing

The clinically established safe dosing regimen of RMC-035 as assessed in healthy subjects is 2.6 mg/kg as single dose (SAD study) and 1.3 mg/kg, administered 6 times in 48 hours, as a multiple dose (MAD study).

RMC-035 has lower clearance in renally impaired subjects resulting in higher AUC (Study 19ROS-03), ie, AUC is approximately doubled when eGFR is reduced by 50%.

In a Phase 1b study in cardiac surgery subjects (20-ROS-04), RMC-035 was administered 5 times in 48 hours. The first dose was 1.3 mg/kg administered over a one-hour infusion, and subsequent doses were 1.3 mg/kg administered during 30 minutes at 6, 12, 24 and 48 hours after the start of the first administration. The start dose was 1.3 mg/kg if pre-surgery eGFR>60 mL/min/1.73m^2 and 0.65 mg/kg if pre-surgery eGFR ≥30 and <60 mL/min/1.73m^2. Protocol-guided dose adjustments and/or IMP discontinuation criteria were also imposed to limit cumulative exposure due to renal impairment. The resulting median C_{max} were well below the C_{max} at the most conservatively set NOAEL (in the marmoset). The median $AUC_{0\text{-}24h}$ were close to or below the $AUC_{0\text{-}24h}$ at the NOAEL in the marmoset and cynomolgus, respectively. By protocol design, these exposure levels are not predicted to be exceeded in the phase 2 study (20-ROS-05).

In summary, the dose/dosing regimen in the planned Study 21-ROS-05 has been carefully determined considering available clinical PK and safety data in healthy subjects, renally impaired subjects and cardiac surgery patients. A 5-dose regimen for this first efficacy study of RMC-035 is selected based on its mode-of-action and relevant disease mechanisms in CS-AKI and is considered to provide relevant intra-and post-operative renal protection of RMC-035. It has been conservatively guided by preceding nonclinical and clinical studies including the cardiac surgery study 20-ROS-04 with a similar dosing regimen. Please see Section 2.1.3.4 on PK and safety data from study 20-ROS-04. The predicted exposures in the planned Study 21-ROS-05 are pharmacologically active and within the clinically safe exposure range, including preservation of relevant nonclinical exposure margins.

For further details, please see the Investigator's Brochure.

6.3. Dose Adjustment Criteria

Dose adjustment will not be permitted throughout the study.

6.4. Treatment Compliance

When subjects are dosed at the investigational site, they will receive IMP directly from the Investigator or designee, under medical supervision to guarantee treatment compliance. The date and start and stop time of each infusion as well as the volume administered will be recorded in the source documents and transcribed to the CRF.

6.5. Randomization and Blinding

Each subject who enters the screening period, which starts when the subject signs the ICF, will receive a unique subject identification number. This number will be used to identify the subject throughout the study and must be used on all study documentation related to the subject.

Eligible subjects will be randomized prior to surgery to receive either RMC-035 or placebo in a 1:1 randomization ratio using a centralized randomization process. Both region (North America and Europe) and Day -1 eGFR calculated using 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.

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.

6.5.1. Breaking the Blind

The treatment code for a given randomized subject will be provided in the event of a medical emergency requiring knowledge of the treatment assigned to the subject. The time, date, subject number and reason for obtaining any of these codes, and therefore breaking the blind, must 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 other study personnel will be made aware of the treatment given unless a medical emergency necessitates such disclosure.

Except for determining whether a Suspected Unexpected Serious Adverse Reaction (SUSAR) requires expedited reporting to regulatory authorities, unblinding of the study medication should only be considered for subject safety or when critical therapeutic decisions are contingent upon knowing the blinded IMP assignment. Any unblinding by the investigational staff must be reported immediately to the Sponsor or designee and must include an explanation of why the study medication was unblinded. If possible, the Sponsor and/or designee should be contacted prior to unblinding of the study medication.

7. INVESTIGATIONAL MEDICINAL PRODUCT MATERIALS AND MANAGEMENT

7.1. Investigational Medicinal Product

The IMP, RMC-035 and placebo, are described in Sections 6.1.1 and 6.1.2, respectively. Refer to the Pharmacy Manual for detailed instructions on preparation, handling, storage and accountability of the IMP.

7.2. Investigational Medicinal Product Packaging and Labelling

RMC-035 concentrate for solution for infusion and its corresponding placebo are filled in glass vials each containing an extractable volume of 5 mL solution. The vials will be packaged in a suitable carton box.

RMC-035 concentrate for solution for infusion and placebo are visually identical. Vials of each kind will be labelled and packaged separated from each other in a non-blinded way. Blinding will be assured at the time of labelling of the infusion syringe.

The IMP will be released to the site upon receipt of all required essential documents. All medication provided to the study site will be prepared, packaged, and labelled by the Sponsor according to Standard Operating Procedures (SOPs), Good Manufacturing Practice guidelines, International Conference on Harmonisation Good Clinical Practice guidelines (ICH GCP), and applicable local laws/regulations.

7.3. Investigational Medicinal Product Storage

Vials with RMC-035 and vials with placebo must be stored at -20 ± 5°C (-4°F) in a secure location with controlled access.

The Investigator or designee must confirm appropriate temperature conditions have been maintained during transit for all IMPs received and that any discrepancies are reported and resolved before use of the study product.

The storage temperature must be monitored by a calibrated, stationary and continuously recording system. Minimum requirement is a calibrated min/max thermometer.

A temperature log must be kept to document storage within the correct temperature interval. Centralized monitoring and alerting system should be used or the temperature should be checked at least every working day. Any deviations in storage temperature must be reported immediately to Sponsor and the IMP must not be used until acceptance from the Sponsor is received.

7.4. Investigational Medicinal Product Preparation

Preparation of the IMP will be done by trained staff in a dedicated area, as detailed in the Pharmacy Manual.

The IMP will be diluted with 0.9% sodium chloride to a volume of 50 mL prior to administration. The drug concentration of the prepared solution for IV infusion will depend on the body weight-adjusted dose for each subject. The pre-surgery body weight collected on Day -1 (Visit 1) should be used to calculate the dose for all 5 dose administrations.

The diluted product may be stored in a prefilled infusion syringe in a refrigerator (2-8°C / 36-46°F) for a maximum of 72 hours (or a shorter time period as specified in the Pharmacy Manual, if applicable). Administration to the subject shall be completed within 2 hours after the syringe has been taken out of the refrigerator.

7.5. Administration

Only subjects enrolled in the study may receive IMP and only trained and authorized site staff may prepare or administer it.

IMP will be administered five (5) times as an IV infusion with a syringe infusion pump. Syringes with 50 mL of solution, specifically diluted and labelled in a blinded manner for the specific subject, will be delivered to the staff at each investigational site.

The IMP should be administered via a CVC whenever possible. One lumen of the CVC should be reserved for IMP administration in order to avoid potential interactions. If a CVC is not available, IMP may also be delivered in a peripheral venous line, specifically reserved for IMP infusion.

First IMP administration will start during surgery, approximately 10 minutes before the expected initiation of CPB. The first infusion and second infusion will be administered IV over approximately 60 minutes (infusion rate of 0.83 mL/min). The start time of the first dose administration will serve as reference point for the 4 remaining dose administrations and protocol-mandated assessments. The second infusion will be administered at 6 hours after the start of the first infusion. The remaining 3 doses will be given as IV infusions, each over approximately 30 minutes (infusion rate of 1.67 mL/min), at 12, 24 and 48 hours respectively after start of the first dose administration.

In case the subject is discharged from the ICU to a non-ICU ward prior to last administration, the remaining dose(s) will be administered either by trained non-ICU staff, or preferably, by trained ICU staff at the non-ICU ward.

All administrations will be done under medical supervision.

Refer to the Pharmacy Manual for detailed guidance for administration of the IMP including use of syringe pump and auxiliary supplies.

7.6. Investigational Medicinal Product Accountability

The pharmacy and the investigational site will maintain appropriate Storage and Accountability logs as well as a Subject Dispensing Log or equivalent detailing dates and quantities of IMP received, prepared for and used by each subject.

7.7. Investigational Medicinal Product Handling and Disposal

All used, partly used, expired or damaged IMP must be stored separately from non-allocated study products. No temperature monitoring is required. Destruction of used and partly used IMP in vials and infusion syringes can be performed on an ongoing basis according to local procedures. Before destruction of vials, appropriate accountability of vials and reconciliation by the monitor should be performed. Documentation on destruction will be forwarded to the Sponsor.

Any unused IMP will be returned to the Sponsor at the conclusion of the study or destroyed according to the clinical site's SOP, as mutually agreed upon by the Sponsor and the site, after drug accountability has been performed and verified by the monitor.

8. STUDY ASSESSMENTS AND PROCEDURES

Study assessments and procedures are described below. All assessments are to be performed according to the timing and frequency described in the SOA (Table 3).

8.1. Informed Consent

The nature of the risks and benefits associated with participation in the study will be explained to all potential study subjects. Written informed consent (including consent for the use and disclosure of research related health information) must be obtained before the subject can begin any screening procedures that are not considered standard patient care. See Section 13.3 for details on the consenting process.

8.2. Demographic Data

Demographic data will be collected and will include gender, age, race, and ethnicity.

8.3. Medical and Surgical History

Medical/surgical history and disease characteristics, a listing of relevant past diseases (within 5 years prior to Screening) and current diseases (ongoing at the time of Screening), and the details of active diseases will be obtained from medical records and recorded in the CRF.

8.4. Prior and Concomitant Medications

Medications are classified as prior if the stop date is before or on the day the ICF is signed.

Prior prescription and non-prescription medications (eg, over-the-counter drugs and herbal supplements) taken within 30 days prior to scheduled date of surgery up to the date of consent will be obtained by the Investigator or designee by interview and/or medical history.

Concomitant medications refer to all drugs and therapies used from the time the ICF was signed through the end of study participation and are described in Section 6.1.3.

All medications (including anesthesia) given before and during surgery will be recorded and transferred to the CRF.

The Sponsor should be contacted if there are any questions regarding concomitant or prior therapy.

8.5. Weight and Height

Weight and height will be collected at screening. Weight will be collected at all timepoints as noted in the SOA. During the subject's stay at the ICU, collection of weight is required only if possible. The pre-surgery weight taken on Day -1 will be used to calculate the IMP doses for all 5 infusions.

8.6. Safety Assessments

Any abnormalities in the safety assessments will be specified and documented as clinically significant or not clinically significant by the Investigator. Abnormal post-dose findings assessed by the Investigator as clinically significant will be reported as AEs.

Any abnormal laboratory test results (hematology, clinical chemistry, liver function or urinalysis) or other safety assessments (eg, ECG, vital signs measurements), including those that worsen from the screening visit, considered clinically significant in the medical and scientific judgment of the Investigator (ie, not related to progression of underlying disease or medical procedure, ie, cardiac surgery) is an AE (Section 9.1).

8.6.1. Assessments Before, During and After Surgery

Specific surgery assessments performed before, during and after surgery will be collected as per the standard hospital care and transferred to the CRF.

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 of bypasses performed
- Length of time with mean arterial pressure <50 mmHg
- Valve surgery type (replacement or repair), replacement valve origin (bioprosthetic or mechanical), and aortic repair type
- Time of admission to the ICU.

8.6.2. Physical Examination

The Investigator or qualified designee will conduct the exams, determine findings, and assess any abnormalities as to clinical significance. The physical examination performed at screening should be comprehensive; all other physical examinations may be abbreviated and symptom driven.

8.6.3. Vital Signs

Vital signs will include body temperature (°C), systolic and diastolic blood pressure (mmHg), heart rate (bpm) and respiratory rate (breaths per min), and oxygen saturation (SpO2).

Vital signs should when possible be measured after a period of at least 5 minutes of rest and should be collected prior to blood draw for laboratory tests. Blood pressure should be recorded in a supine position when possible. Blood pressure, heart rate, and respiratory rate measurements will be assessed using either manual techniques or an automated device.

8.6.4. Electrocardiograms

A single 12-lead electrocardiogram (ECG) will be obtained in supine position using an ECG machine that automatically calculates the heart rate and measures PR, QRS, QT, and QTc intervals. Twelve-lead ECGs will be reviewed and assessed for clinical significance by the Investigator. Other cardiac monitoring, eg, telemetry, will follow local standard of care and not recorded in the CRF.

8.7. Laboratory Assessments

The samples will be collected at the time points indicated in the SOA (Table 3). Actual sampling times will be recorded in the CRF. A list of clinical laboratory tests to be processed by the local laboratory is provided in Table 5. A list of clinical laboratory tests to be processed at the central laboratory is provided in Table 6.

Table 5: Protocol Required Laboratory Assessments – Local Laboratory

Laboratory Assessments	**Parameters**		
Hematology	Hematocrit (Hct)	Red cell distribution width (RDW)	Monocytes
	Hemoglobin (Hb)	Red blood cells	Lymphocytes
	Mean corpuscular volume (MCV)	Platelets	Eosinophils
	Mean corpuscular hemoglobin (MCH)	Leucocytes	Basophils
	Mean corpuscular hemoglobin concentration (MCHC)	Neutrophils	
Clinical Chemistry (excluding liver function labs)	Albumin	Sodium	Blood urea nitrogen
	Calcium	Estimated glomerular filtration rate (based on Serum creatinine)	Uric acid
	Chloride	Magnesium	Glucose
	Serum creatinine (SCr)	Phosphate	
	C-reactive protein (CRP)	Potassium	
Liver Function Labs	Alanine aminotransferase (ALT)	Aspartate aminotransferase (AST)	Gamma glutamyltransferase (GGT)
	Alkaline phosphatase (ALP)	Bilirubin (total and conjugated)	
Urinalysis	Specific gravity	pH	Leukocytes
	Erythrocytes	Protein	Glucose

Laboratory Assessments	Parameters		
	Nitrite	Urobilinogen	Bilirubin
	Ketones	Microscopic examination (if blood or protein is abnormal)	
Urine albumin creatinine ratio	Urine albumin	Urine creatinine	
Other Screening Tests	Highly sensitive serum human chorionic gonadotropin (hCG) serum pregnancy test (as needed for WOCBP)		

Table 6: Protocol Required Laboratory Assessments – Central Laboratory

Laboratory Assessments	Parameters		
Clinical Chemistry	Serum creatinine (SCr)	Cystatin C	
Urine albumin creatinine ratio (UACR), Urine protein creatinine ratio (UPCR)	Urine albumin	Urine creatinine	Urine protein
Urine Kidney Biomarkers	KIM-1, NGAL, TIMP2, IGFBP7, CCL-14, IL-18, LFABP, 8-OHdG, and urine creatinine		
Cardiac Biomarkers	NT-pro-BNP	Troponin-I (cTnI)	Troponin T (cTnT).
Immunologic biomarkers	Parameters characteristic for formation of including but not limited to markers of complement activation, cytokine release and mast cell activation		
Pharmacokinetics	Concentration of RMC-035 in plasma		
ADA	Determination of ADAs in plasma		

Abbreviations: 8-OHdG=8-hydroxy-2'-deoxyguanosine; CCL-14=chemokine ligand 14; IGFBP7=insulin-like growth binding factor protein 7; IL-18=interleukin-18; KIM-1=kidney injury molecule 1; LFABP=liver fatty acid binding protein; NGAL=neutrophil gelatinase-associated lipocalin; NT-pro-BNP= N-terminal-pro-b-type natriuretic peptide; TIMP2=tissue inhibitor of metalloproteinase 2

Blood samples for analysis of clinical chemistry (including SCr, cystatin C and liver function tests), cardiac and immunologic biomarkers and hematology parameters will be collected through venipuncture or an indwelling venous or arterial catheter.

Blood samples will be analyzed for SCr both locally and centrally; local results will be utilized for eligibility, randomization / stratification, IMP dose assignment, clinical care and real time evaluation of AKI for purposes of IMP discontinuation (Section 5.3.1), while centrally processed blood samples will be utilized for purposes of endpoint evaluation.

The Investigator must review the laboratory reports, document the result of the review (ie, clinical assessment for values out of range) on the laboratory report, and record any clinically significant changes occurring during the study in the AE section of the CRF. The laboratory reports must be filed with the source documents.

Clinically significant abnormal laboratory findings are those which are not associated with the underlying disease, unless judged by the Investigator to be more severe than expected for the subject's condition.

All laboratory tests which are considered to be clinically significant by the Investigator during the study should be repeated until the values return to normal or baseline or are no longer considered clinically significant by the Investigator or medical monitor. If such values do not return to normal/baseline within a period of time judged reasonable by the Investigator, the etiology should be identified and the Sponsor notified.

If laboratory values from non-protocol specified laboratory assessments performed at the institution's local laboratory require a change in subject management or are considered clinically significant by the Investigator (eg, SAE or AE), then the results must be recorded in the CRF.

8.7.1. Urine Albumin Creatinine Ratio and Urine Protein Creatinine Ratio

The UACR screening sample (Day -1, Visit 1) 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).

The 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 via central lab only.

Follow-up samples (Day 30, Visit 7 and Day 90, Visit 8) will be collected preferably as FMV samples and analyzed in a central lab only. If FMV is not feasible, spot urine sample should be collected in its place.

Detailed instructions for urine collection protocol will be provided in the laboratory manual.

8.7.2. Urinalysis

Urinalysis by means of a regular dipstick test will be performed locally.

8.7.3. Pregnancy Testing

All female subjects of child-bearing potential will have a serum pregnancy test performed at Screening; pregnant subjects are excluded from participation in the study. A serum pregnancy test completed within 48 hours prior to surgery does not need to be repeated on the day of surgery. If the screening visit 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.

8.8. Urine Output

Urine output (UO) will be documented starting on Day 1 from the insertion of the Foley catheter and continue for as long as the subject has a catheter (Foley) in place, or until 72 hours after the first dose of IMP (whichever occurs first).

While the subject remains hospitalized in the ICU, site staff will measure UO in hourly intervals or per site’s standard of care. Urine output (volume) will be entered into the CRF at 6-hour time periods as much as possible. The exact time period for the collection and the collected volume will be recorded in the CRF.

If the subject is transferred to a non-ICU ward, and the catheter is still in place, UO will be measured at 6-hour intervals or per site's SOC, until catheter is removed or until 72 hours after the first dose of IMP. The exact time period for the collection and the collected volume will be recorded in the CRF at 6-hour intervals as much as possible.

If the Foley catheter is removed, urine production will no longer be entered in the CRF but spot urine samples will continue to be collected as per the SOA. UO can only be used for diagnosing AKI or rating AKI severity if the UO has been calculated based on urine output measured by a Foley catheter. If the Foley catheter is removed, UO will not be used for the AKI definition.

8.9. Major Adverse Kidney Events

Major adverse kidney events (MAKE) will be assessed at Days 30 and 90. The MAKE endpoint is a composite of death, new RRT (any RRT after surgery), or sustained loss of kidney function, defined as a 25% or greater decline in eGFR as it represents a clinically meaningful endpoint as suggested by the National Institutes of Diabetes and Digestive and Kidney Diseases .

8.10. Pharmacokinetic Assessments

Blood samples for the determination of plasma concentrations of RMC-035 after administration of the IMP will be collected through a central or peripheral line in relation to IMP dose 1, 4 and 5.

The actual date and time (24-hour clock time) of each sample will be recorded and transferred to the CRF.

Samples will be taken at the pre-specified time points described in Table 7.

Table 7: Plasma Pharmacokinetic Sampling Timepoints

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

Abbreviations: PK=pharmacokinetic; t=time; min=minutes; h=hour

Drug concentration information that may unblind the study will not be reported to investigational site or blinded personnel until the study has been unblinded. Both the bioanalytical laboratory and the pharmacokineticist will be blinded to treatment.

Samples may be stored at the analytical laboratory until the database lock has been performed.

8.11. Immunogenicity Assessments

Blood samples for the determination of ADA after administration of IMP will be collected through an indwelling arterial or venous catheter or venipuncture at all timepoints as described in the SOA and will be processed via central lab.

Instruction on the sample collection and handling will be detailed in the Laboratory Manual.

Initial screening of ADA will be performed using a standard bridging assay. Positive results in the screening assay will be confirmed by elimination of false positive results considering the limitations of the screening assay.

Additionally, serum samples should also be collected at the final visit from subjects who discontinued study intervention or were withdrawn from the study.

Samples may be stored for a maximum of 2 years (or according to local regulations) following the last subject's last visit for the study at a facility selected by the Sponsor to enable further analysis of immune responses to the IMP.

Potential ADAs will be further characterized with regard to Ig subtype, neutralizing capacity and cross-reactivity with endogenous A1M.

Assessment of exploratory immunologic biomarkers investigating the background of potential IRRs is described in section 8.13.2.

8.12. Health-Related Quality of Life Assessments

Two patient recorded outcome (PRO) questionnaires will be collected at all timepoints as described in the SOA (during screening and Visit 8, Day 90). Questionnaires should be completed by the subjects themselves and should be administered and completed prior to any study specific procedures on the specific visit days. In the event that a subject has limitations that preclude them from completing the questionnaire, the questionnaire may be completed for them, based on their verbal responses by study personal and/or legally acceptable representative following questionnaire administration instructions outlined in the Study Manual.

Medical Outcomes Study (MOS) 36-Item Short Form Health Survey (SF-36)

The MOS SF-36 (see Appendix 4) is a set of generic, coherent, and easily administered Quality of Life measures. The SF-36 includes one multi-item scale that assesses eight health concepts:

- limitations in physical activities because of health problems
- limitations in social activities because of physical or emotional problems
- limitations in usual role activities because of physical health problems
- bodily pain
- general mental health (psychological distress and well-being)
- limitations in usual role activities because of emotional problems
- vitality (energy and fatigue)
- general health perceptions

European Quality of Life 5 Domain 5-Level Score (EQ-5D-5L)

The European Quality of Life 5 Domain 5-Level Score (EQ-5D-5L) (see Appendix 5) is a self-report survey that measures quality of life across 5 domains: mobility, self-care, usual activities, pain/discomfort, and anxiety/depression. The EQ-5D-5L is consists of two parts: the EQ-5D descriptive system and the EQ visual analogue scale (EQ VAS).

The descriptive system comprises five dimensions: mobility, self-care, usual activities, pain/discomfort and anxiety/depression. Each dimension has 5 levels: no problems, slight problems, moderate problems, severe problems and extreme problems. The subject is asked to indicate his/her health state by ticking the box next to the most appropriate statement in each of the five dimensions. This decision results in a 1-digit number that expresses the level selected for that dimension. The digits for the five dimensions can be combined into a 5-digit number that describes the subject's health state.

The EQ VAS records the subject's self-rated health on a vertical visual analogue scale, where the endpoints are labelled "The best health you can imagine" and "The worst health you can imagine." The VAS can be used as a quantitative measure of health outcome that reflect the subject's own judgement.

8.13. Exploratory Biomarkers

Plasma and urine samples for kidney, cardiac and immunologic biomarker assessment will be collected as per the SOA.

Samples may be stored for a maximum of 2 years (or according to local regulations) following the last subject's last visit for the study at a facility selected by the Sponsor to enable further analysis of pharmacodynamic and immunologic responses to the IMP.

8.13.1. Kidney and Cardiac Biomarkers

Blood and urine samples for kidney and cardiac biomarker research will also be collected at all timepoints as described in the SOA.

- Blood samples will be collected through an indwelling arterial or venous catheter.
- Spot samples of urine where urine is drawn directly from the bladder while the subject is being catheterized and from normal voids once the catheter is removed.

Urine samples will be assessed for KIM-1, NGAL, TIMP2, IGFBP7, CCL-14, IL-18, LFABP, 8-OHdG, and urine creatinine. Plasma samples will be assessed for NT-pro-BNP and cardiac troponin I and T (cTnI, cTnT).

Additional biomarkers may be measured from already collected samples.

8.13.2. Immunologic Biomarkers

Additional plasma samples will be collected at all timepoints as described in the SOA to explore the background and mechanism of potential IRRs.

9. SAFETY MONITORING AND REPORTING

9.1. Definition of an Adverse Event

An AE is any untoward medical occurrence in a clinical study subject, temporally associated with the use of medicinal product, whether or not considered related to this IMP. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of medicinal product.

By definition, a treatment-emergent adverse event (TEAE) is any AE that occurs after the initial IMP administration through 72 hours (inclusive) after the final IMP administration.

Events Meeting the AE Definition

- Any abnormal laboratory test results or other safety assessments including those that worsen from baseline, considered clinically significant in the medical and scientific judgment of the Investigator (ie, not related to progression of underlying disease or surgical procedure).
- Exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency and/or intensity of the condition.
- New conditions detected or diagnosed after IMP administration even though it may have been present before the start of the study.
- Signs, symptoms, or the clinical sequelae of a suspected drug-drug interaction.
- Signs, symptoms, or the clinical sequelae of a suspected overdose of either IMP or a concomitant medication. Overdose per se will not be reported as an AE/SAE unless it is an intentional overdose taken with possible suicidal/self-harming intent. Such overdoses should be reported regardless of sequelae.

Events NOT Meeting the AE Definition

- Any clinically significant abnormal laboratory findings or other abnormal safety assessments which are associated with the underlying disease or the surgical procedure, unless judged by the Investigator to be more severe than expected for the subject's condition.
- The disease/disorder being studied or expected progression, signs, or symptoms of the disease/disorder being studied, unless more severe than expected for the subject's condition.
- Medical or surgical procedure (eg, endoscopy, appendectomy): the condition that leads to the procedure is the AE.
- Situations in which an untoward medical occurrence did not occur (social and/or convenience admission to a hospital).
- Anticipated day-to-day fluctuations of pre-existing disease(s) or condition(s) present or detected at the start of the study that do not worsen.

9.2. Definition of a Serious Adverse Event

An SAE is defined as an AE that meets at least one of the following serious criteria:

- **Results in death**
- **Is life-threatening**
    - The term “life-threatening” in the definition of “serious” refers to an event in which the subject was at risk of death at the time of the event. It does not refer to an event, which hypothetically might have caused death, if it were more severe.
- **Requires inpatient hospitalization or prolongation of existing hospitalization**
    - In general, hospitalization signifies that the subject has been detained (usually involving at least an overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or outpatient setting. Complications that occur during hospitalization are AEs. If a complication prolongs hospitalization or fulfills any other serious criteria, the event is serious. When in doubt as to whether "hospitalization" occurred or was necessary, the AE should be considered serious.
    - Hospitalization for elective treatment of a pre-existing condition that did not worsen from baseline is not considered an AE.
- **Results in persistent disability/incapacity**
    - The term disability means a substantial disruption of a person's ability to conduct normal life functions.
    - This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhea, influenza, and accidental trauma (eg, sprained ankle) which may interfere with or prevent everyday life functions but do not constitute a substantial disruption.
- **Is a congenital anomaly/birth defect**
- **Other medically important serious event**
    - Medical or scientific judgment should be exercised in deciding whether SAE reporting is appropriate in other situations such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the subject or may require medical or surgical intervention to prevent one of the other outcomes listed in the above definition. These events should usually be considered serious.
    - Examples of such events include invasive or malignant cancers, intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse.

9.2.1. Suspected Unexpected Serious Adverse Reactions

Suspected unexpected serious adverse reactions (SUSARs) are serious adverse events that are not listed in the IB and the Investigator or Sponsor identifies as related to investigational product

or procedure. The Sponsor or designee has procedures in place for the expedited reporting of SUSARS that are consistent with global regulations and associated guidelines.

9.3. Adverse Events of Special Interest

Injection Site Reaction (ISR) and Infusion Related Reaction (IRR) of grade 3 or higher are considered Adverse Events of Special Interest and must be reported within 24 hours following the SAE reporting protocol described in Section 9.7. The criteria of intensity grading for ISR and IRR are provided in Table 8 below.

Table 8: Intensity Grading For Injection Site Reactions (ISR) and Infusion Related Reactions (IRR)

Term	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5	Definition
MedDRA PT: Injection site reaction MedDRA SOC: General disorders and administration site conditions	Tenderness with or without associated symptoms (eg, warmth, erythema, itching)	Pain; lipodystrophy; edema; phlebitis	Ulceration or necrosis; severe tissue damage; operative intervention indicated	Life-threatening consequences; urgent intervention indicated	Death	A disorder characterized by an intense adverse reaction (usually immunologic) developing at the site of an injection.
MedDRA PT: Infusion related reaction MedDRA SOC: Injury, poisoning and procedural complications	Mild transient reaction; infusion interruption not indicated; intervention not indicated	Therapy or infusion interruption indicated but responds promptly to symptomatic treatment (eg, antihistamines, NSAIDS, narcotics, IV fluids); prophylactic medications indicated for ≤24 hrs	Prolonged (eg, not rapidly responsive to symptomatic medication and/or brief interruption of infusion); recurrence of symptoms following initial improvement; hospitalization indicated for clinical sequelae	Life-threatening consequences; urgent intervention indicated	Death	A disorder characterized by adverse reaction to the infusion of pharmacological or biological substances.

Abbreviations: IV=intravenous; MedDRA=Medical Dictionary for Regulatory Activities; NSAID=non-steroidal anti-inflammatory drug; SOC=system organ class

9.4. Relationship to Investigational Medicinal Product

The investigator is obligated to assess and record the relationship between the IMP and each occurrence of each AE/SAE according to the following definitions:

- **Not related (unlikely)**
    - Does not follow a reasonable temporal sequence from drug administration

  - Is readily explained by the subject's clinical state or by alternative etiologies such as underlying disease(s), concomitant therapy and other risk factors

- **Related (possible/probable)**
  - Follows a reasonable temporal sequence from IMP administration
  - Cannot be reasonably explained by the known characteristics of the subject's clinical state

Alternative etiology should be provided for all AEs assessed as possibly related to IMP.

The Investigator will use clinical judgment to determine the relationship.

Alternative causes, such as underlying disease(s), concomitant therapy, and other risk factors, as well as the temporal relationship of the event to study intervention administration will be considered and investigated. The Investigator will also consult the IB and any updates in his/her assessment.

For each AE/SAE, the Investigator must document in the medical notes that he/she has reviewed the AE/SAE and has provided an assessment of causality.

There may be situations in which an SAE has occurred and the Investigator has minimal information to include in the initial report. However, it is very important that the Investigator always assess causality for every event before the initial transmission of the SAE data to the Sponsor or CTI Safety.

The investigator may change his/her opinion of causality in light of follow-up information and send a SAE follow-up report with the updated causality assessment.

The causality assessment is one of the criteria used when determining regulatory reporting requirements.

9.5. Assessment of Intensity

Intensity will occur according to the following scale:

Grade 1 Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.

Grade 2 Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental Activities of Daily Living (ADL).

Grade 3 Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL.

Grade 4 Life-threatening consequences; urgent intervention indicated

Grade 5 Death related to AE

Instrumental ADL refers to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc. Self-care ADLs refer to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.

It is important to distinguish between serious and severe AEs. Severity is a measure of intensity whereas seriousness is defined by the criteria under Section 9.2. An AE of severe intensity may not be considered serious.

9.6. Recording Adverse Events

All AEs will be collected from initiation of the first IMP administration through the Day 30 study visit.

All SAEs will be collected from the signing of the ICF through the EOS visit.

When an AE/SAE occurs, it is the responsibility of the Investigator to review all documentation (eg, hospital progress notes, laboratory reports, and diagnostics reports) related to the event. The AE/SAE term should be reported in standard medical terminology when possible. For each event, the Investigator will evaluate and report the onset, resolution, intensity, causality, action taken, serious outcome (if applicable), and whether or not it caused the subject to discontinue the study.

The Investigator must judge outcome of the AE by the following terms:

- **Recovered**
  - fully recovered, or by medical or surgical treatment the condition has returned to the level observed at the first trial related activity after the subject signed the informed consent.
- **Recovering**
  - the condition is improving and the subject is expected to recover from the event. This term should only be used when the subject has completed the trial.
- **Recovered with sequelae**
  - as a result of the AE the subject suffered persistent and significant disability/incapacity (eg, became blind, deaf, paralyzed). If the sequelae meet seriousness criteria, the AE must be reported as an SAE.
- **Not recovered**
- **Fatal**
- **Unknown**
  - should only be used in cases where the subject is lost to follow-up.

Any requested supporting documentation (eg, laboratory or diagnostic test results, clinical or progress notes, admission and/or discharge records) requested by the Sponsor or CTI Safety, related to SAEs for the evaluation of safety must be faxed or emailed to CTI Safety as soon as available at the study site. Specific email address and fax numbers and instructions will be specified in the Study Manual.

If a subject dies during participation in the study or during a recognized follow-up period, the Investigator will provide the Sponsor or designee with a copy of any post-mortem findings including histopathology.

9.7. Reporting Adverse Events

All AEs must be recorded in the electronic data capture (EDC) system in a timely manner and will be described by seriousness, duration (event start and resolution), severity, outcome, action taken, and relationship to investigational product. The Investigator should take all appropriate measures to ensure the safety of the subjects and should follow-up the outcome of any AE (clinical, laboratory values, or other, etc.) until the return to normal, until the subject's condition has stabilized, or the event is chronic. In the event of an SAE, the Investigator should follow-up

with the outcome until the clinical recovery is complete and laboratory results have returned to normal or until progression has been stabilized.

All SAEs, regardless of causality, must be promptly recorded in the EDC system within 24 hours of learning of its occurrence. If the site experiences a temporary disruption of the EDC system, a back-up paper SAE report form will be available for completion and is located in The Study Manual.

Additional follow-up information must also be recorded in the EDC system within 24 hours of awareness.

The Investigator must also report all SAEs promptly to the appropriate IRB/IEC as required.

Investigators are not obligated to actively seek AEs or SAEs in former study subjects. However, if the Investigator learns of any SAE, including a death, at any time after a subject has been discharged from the study, and he/she considers the event to be reasonably related to the IMP or study participation, the Investigator must promptly notify the Sponsor.

9.8. Pregnancy and Lactation

Refer to Appendix 1 for more details on contraceptive guidance and collection of pregnancy information.

There is no relevant clinical experience with RMC-035 in pregnant or lactating women. Women of child-bearing potential must have a negative pregnancy test prior to enrollment because of the unknown risk of potential effects on the fetus. Women of childbearing potential should be monitored according to local and country-specific regulations. This IMP should not be administered to pregnant women or women who are breastfeeding. Any female subject who becomes pregnant while participating in the study will discontinue IMP administration.

Female subjects of childbearing potential are required to use highly effective contraception from screening up to 7 days after the final IMP administration. Male subjects with female partners of childbearing potential must be using a condom starting at screening and continue to do so throughout the treatment period and for 7 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. Refer to Appendix 1 for a complete list of highly effective contraception methods.

Pregnancy in itself is not regarded as an AE unless there is suspicion that the investigational product may have interfered with the effectiveness of a contraceptive medication. However, complications of pregnancy and abnormal outcomes of pregnancy are AEs and may meet serious criteria for an SAE (eg, ectopic pregnancy, spontaneous abortion, intrauterine fetal demise, neonatal death, or congenital anomaly). Elective abortions without complications should not be reported as AEs.

A pregnancy or positive pregnancy test must be recorded immediately using the Pregnancy Report Form in the EDC system. The Investigator must follow-up and document in the EDC system the course and outcome of all pregnancies even if the subject discontinued from the study.

9.9. Data Monitoring Committee

An Independent DMC including clinical expertise in kidney injury will perform review of safety findings including possible adverse drug effects on the kidney 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 their reviews, including the review in relation to interim analysis (see section 10.4.1).

Communication from the DMC will be blinded. All DMC activities and processes will be outlined in the DMC Charter.

10. STATISTICAL CONSIDERATIONS

10.1. Analysis Sets

Intent to Treat (ITT): The ITT will consist of all randomized subjects.

Modified Intent to Treat (mITT) Set: The mITT will consist of all subjects in the ITT set who received at least 1 dose of IMP. The mITT will be used for the primary analysis of efficacy endpoints. Subjects will be included in the efficacy analysis based on randomized treatment group.

Safety Analysis (SAF) Set: The SAF will consist of all subjects in the mITT set; however, subjects will be included in the analysis based on treatment actually received.

Per Protocol (PPS) Set: The PPS will consist of all subjects in the mITT set who did not have any major protocol deviations or other conditions (eg insufficient exposure) that were judged to influence the efficacy analysis. Prior to database lock, subjects who had a major protocol deviation will be identified in a blinded manner by the study team.

PK Analysis (PK) Set: The PK Analysis Set will be used for the PK evaluation and will consist of all randomized subjects with no relevant protocol deviations affecting the evaluation of the PK parameters.

10.2. Sample Size Determination

For the primary endpoint, AKI 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%. A sample size of 268 subjects dosed leads to a test power of 80% to show statistically significant results at a two-sided significance level (alpha) of 0.10. An IA of the primary endpoint will be performed when 50% of the planned subjects dosed have completed Visit 6 (Day 5-9). Conditional power (CP) given ‘the current trend’ will be calculated. Sample size may be increased to a maximum of 348 subjects dosed. Sample size will not be decreased.

10.3. Stratification and Randomization

Eligible subjects will be randomized to receive either RMC-035 or placebo in a 1:1 randomization ratio. Both region (North America and Europe) and Day -1 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.

10.4. Interim Analyses

10.4.1. Primary Endpoint

An interim analysis (IA) of the primary endpoint will be performed when 50% of the planned randomized subjects have completed Visit 6 (Day 5-9). The analysis will be performed as detailed in Section 10.5.2. Conditional power (CP), the probability that the final analysis will be statistically significant, will be calculated. Sample size may be increased to a maximum of 348 randomized subjects. Sample size will not be decreased. The Study may be stopped at the IA for futility reasons. Study eligibility criteria may also be modified.

Given the unblinded nature of this IA, an unblinded Data Monitoring Committee (DMC) of independent individuals not involved in any other study activities will be utilized. Further details on the IA and the control of the type I error will be provided in the DMC Charter and Statistical Analysis Plan.

The Sponsor and all other CRO personnel will remain blinded. The unblinded materials, data, and analysis results will be stored securely and separately from blinded clinical material and will not be accessible by blinded personnel. Results of the IA will not be shared with the Sponsor or blinded CRO personnel; only a decision on whether sample size expansion criteria have been met will be shared with the blinded team.

Control of the type I error will be done by adjusting the final critical value as proposed by Gao et al. (Gao 2008) and further developed by Mehta and Pocock (Mehta 2011). 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).

10.4.2. Exploratory Kidney and Cardiac Biomarkers

An IA of exploratory biomarkers from urine or blood may be performed at a time point determined by the Sponsor. The purpose is providing background information on the mechanism of action and to help guiding additional development programs for RMC-035.

This analysis, if conducted, is not part of, and is run independently from, the IA of the primary endpoint described in section 10.4.1. The analysis will be conducted as detailed in section 10.5.6. The unblinded materials, data, and analysis results will be stored securely and separately from blinded clinical material. In order to minimize impact on the scientific integrity of the study, access to these unblinded data on an individual subject level will be restricted to Sponsor or CRO personnel not involved in the conduct or supervision of the study. A Data Access Plan will detail which personnel will have access to unblinding subject level data.

10.5. Planned Method of Data Presentation and Analysis

The primary analysis will be performed after the last subject has completed the last study visit. Full details of the statistical analyses to be performed will be included in the SAP.

All continuous data will be summarized using N, mean, standard deviation (SD), minimum, median and maximum value. Summary statistics for categorical variables will contain count and percentage. For log-normal endpoints, geometric mean and geometric standard deviation will also be produced.

10.5.1. Demographic and Baseline Characteristics

Demographic data, medical and surgical history, risk factors for AKI, and other relevant baseline characteristics will be summarized by group using summary statistics.

10.5.2. Efficacy

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 (North America and Europe) and eGFR at Day -1 ($\geq$60 and <60 mL/min/1.73m^2). 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:

- 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 ($\mathbf{p}_{\text{RMC-035}} = \mathbf{p}_{\text{placebo}}$)
- 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 ($\mathbf{p}_{\text{RMC-035}} \neq \mathbf{p}_{\text{placebo}}$)

Secondary continuous efficacy endpoints, that are measures of renal function will be analyzed using robust regression of log-transformed values having sequentially multiply imputed any missing data. 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 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 AUC will be calculated using the right Riemann sum with the dates/times the measurements are planned. The relative difference (RMC-035 vs. placebo) and its 90% confidence interval will also be reported.

Supportive analyses of renal function will be performed 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.

Evaluation of the exploratory endpoints, sensitivity analyses and other continuous secondary endpoints, will be detailed in the SAP.

10.5.3. Subgroup Analyses

Subgroup analyses will be defined prior to database lock in the SAP.

10.5.4. Safety

Safety analyses will be conducted on the safety set. To characterize the safety profile, the number and percentage of subjects with TEAEs and AEs per dose group will be tabulated per System Organ Class (SOC) and Preferred Term (PT) according to the Medical Dictionary for Regulatory Activities (MedDRA). 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 dose group.

10.5.5. 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 at baseline.

10.5.6. Exploratory Biomarkers

Descriptive statistics will be obtained by parameter and visit by dose group. This will be done both for the actual values and the changes from baseline. Statistical testing will be performed for exploratory purposes.

10.5.6.1. Kidney and Cardiac Biomarkers

Serum/plasma and urine samples will be collected and stored for potential future analysis of exploratory biomarkers, eg, urine KIM-1, NGAL, TIMP2, IGFBP7, CCL-14, IL-18, LFABP; and plasma NTpro-BNP, cTnI and cTnI. Additional urine and/or plasma biomarkers related to the mode of action of RMC-035 (eg, endogenous alpha-1-microglobulin) may also be analyzed.

10.5.6.2. Immunologic Biomarkers

Plasma samples will be collected for future analysis of parameters relevant for exploration of potential infusion related reactions.

11. DIRECT ACCESS TO SOURCE DATA/DOCUMENTS

11.1. Study Documents

Source documents are original documents, data and records for which the study data are collected and verified. Example of such source documents may include, but are not limited to, hospital records and subject charts; laboratory, pharmacy, and radiology records; subject diaries; microfiches; correspondence; and death registries. Data entered in the CRF that are transcribed from source documents must be consistent with the source documents or the discrepancies must be explained. The Investigator may need to request previous medical records or transfer records, depending on the study.

The Investigator will maintain a list of qualified staff to whom study responsibilities have been delegated. These individuals who are authorized to fulfil these responsibilities should be outlined and included in the Delegation of Authority Form.

The Investigator and study staff are responsible for maintaining a comprehensive and centralized filing system of all subject records that are readily retrieved to be monitored and or audited at any time by the key sponsor contact, regulatory authorities, and IRB/IECs. The filing system will include at a minimum the following:

- Subject content including ICFs and subject ID lists
- Protocols and protocol amendments, IB, copies of pre-study documentation and all IRB/IEC and sponsor communication
- Proof of receipt, experimental treatment flow records, and experimental product-related correspondence

Original source documents supporting entries into CRFs must be maintained at the site and readily available upon request. No study documents should be discarded without prior written agreement between the Sponsor and the Investigator. Should storage no longer be available to archive source documents or must be moved to an alternative location, the research staff should notify the key sponsor contact prior to the shipping the documents.

11.2. Study Monitoring

Before an investigational site can enter a subject into the study, a representative of the Sponsor or designee may visit the investigational study site to:

- Determine the adequacy of the facilities
- Discuss with the Investigator(s) and other personnel their responsibilities under the protocol as well as the responsibilities of the Sponsor. This will be documented in a Clinical Study Agreement between the Sponsor and the Investigator.

During the study, a monitor from the Sponsor or its designee will have regular contacts with the investigational site and conduct on-site monitoring visits as described in the Study Monitoring Plan. Sponsor approved monitoring and auditing procedures will ensure that the study is conducted in accordance with the protocol and regulatory requirements while ensuring the safety of all study subjects.

The monitor will perform source data verification, including a comparison of the data in the CRF with the subject's medical records at the hospital or clinic, and other records relevant to the study. Source data verification requires direct access to all original records for each subject. The review of medical records will be performed in a manner that ensures subject confidentiality.

Regulatory authorities and the IRB /IEC may request access to all source documents for on-site inspection or an audit. Likewise, the Sponsor or its designee may conduct a quality assurance audit to ensure compliance with GCP and all applicable regulatory requirements. Direct access to source documents must be guaranteed by the Investigator, who will provide support at all times for these activities.

Upon completion of the study the monitor will make a final assessment of the conduct of the study and inventory all clinical supplies to be returned to the Sponsor or designee. Unused or used vials of IMP will be stored until the monitor is able to perform a physical inventory and reconciliation with the drug accountability records. At the completion of this study, all unused or used vials must be returned to the Sponsor, or designee, or if authorized, disposed of at the study site and documented.

11.3. Audits and Inspections

Authorized representatives of the Sponsor, a regulatory authority, an IEC, or an IRB may visit the site to perform audits or inspections, including source data verification. The purpose of a Sponsor audit or inspection is to systematically and independently examine all study-related activities and documents to determine whether these activities were conducted, and data were recorded, analyzed, and accurately reported according to the protocol, ICH GCP, and any applicable regulatory requirements. The Investigator should contact the Sponsor immediately if contacted by a regulatory agency about an inspection.

11.4. Institutional Review Board and Independent Ethics Committee

The Principal Investigator must obtain IRB/IEC approval for the investigation. Initial IRB/IEC approval, and all materials approved by the IRB/IEC for this study including the subject consent form/process must be maintained by the Investigator and made available for inspection.

12. DATA QUALITY ASSURANCE

To ensure compliance with Good Clinical Practices and all applicable regulatory requirements, the Sponsor may conduct a quality assurance audit. See Section 11.3 for more details regarding the audit process.

All subject data relating to the study will be recorded in an eCRF unless transmitted to the Sponsor or designee electronically (eg, laboratory data). The Investigator is responsible for verifying that data entries are accurate and correct by physically or electronically signing the CRF. The Investigator must maintain accurate documentation (source data) that supports the information entered in the CRF and must permit study-related monitoring, audits, IRB/IEC review, and regulatory agency inspections and provide direct access to source data documents.

The Sponsor or designee is responsible for the data management of this study including quality checking of the data. Study monitors will perform ongoing source data verification to confirm that data entered into the CRF by authorized site personnel are accurate, complete, and verifiable from source documents; that the safety and rights of subjects are being protected; and that the study is being conducted in accordance with the currently approved protocol and any other study agreements, ICH/GCP, and all applicable regulatory requirements.

12.1. Inspection of Records

The Sponsor or its designee will be allowed to conduct site visits to the investigation facilities for the purpose of monitoring any aspect of the study. The Investigator agrees to allow the monitor to inspect the drug storage area, IMP stocks, drug accountability records, subject charts and study source documents, and other records relative to study conduct.

12.2. Retention of Records

The Principal Investigator must maintain all documentation relating to the study for a period of 2 years after the last marketing application approval, or if not approved 2 years following the discontinuance of the test article for investigation. If it becomes necessary for the Sponsor or the Regulatory Authority to review any documentation relating to the study, the Investigator must permit access to such records.

12.3. Data Protection

Subjects will be assigned a unique identifier by the clinical site staff. Any subject records or datasets that are transferred to the Sponsor will contain the identifier only; subject names or any information which would make the subject identifiable will not be transferred. The subject must be informed that his/her personal study-related data will be used by the Sponsor in accordance with local data protection law. The level of disclosure must also be explained to the subject. The subject must be informed that his/her medical records may be examined by Clinical Quality Assurance auditors or other authorized personnel appointed by the Sponsor, by appropriate IRB/IEC members, and by inspectors from regulatory authorities.

13. ETHICS

13.1. Ethics Review

A copy of the protocol, ICF, and any additional subject or trial information such as subject recruitment materials must be submitted to each sites respective IRB/IEC for approval. Once approval is obtained from the IRB/IEC, all documents must be provided to the key sponsor contact before subject recruitment can begin.

The Investigator must also receive IRB/IEC approval for all protocol and ICF changes or amendments. Investigators must ensure that ongoing/continuous IRB/IEC approval (ie, annual approval) is provided throughout the conduct of the study. Copies of IRB/IEC approval are to be forwarded to the key sponsor contact for archiving.

During the course of the study, Investigators are to submit site-specific and study SAEs (provided to the site by the key sponsor contact) along with any protocol deviations to their IRB/IEC in accordance with their respective IRB/IEC policies.

13.2. Ethical Conduct of the Study

The study will be performed in accordance with ethical principles that have their origin in the Declaration of Helsinki, Council for International Organizations of Medical Sciences (CIOMS) International Ethical Guidelines and are consistent with applicable ICH/GCP Guidelines, and applicable laws and regulations.

The Investigator will be thoroughly familiar with the appropriate use of the investigational product as described in the protocol and IB. Essential clinical documents will be maintained to demonstrate the validity of the study and the integrity of the data collected. A Trial Master File (TMF) will be established at the beginning of the study, maintained for the duration of the study, and retained according to appropriate regulations.

13.3. Written Informed Consent

The Investigator or his/her representative will explain the nature of the study to the subject or his/her legally authorized representative and answer all questions regarding the study. Subjects must be informed that their participation is voluntary. Subjects or their legally authorized representative will be required to sign a statement of informed consent that meets the requirements of local regulations, ICH guidelines, Personal Information Protection and Electronic Documents Act (PIPEDA) requirements, where applicable, and the IRB/IEC or study center. The subject's signed and dated informed consent will be obtained before conducting any study procedures.

The medical record must include a statement that written informed consent was obtained before the subject was enrolled in the study and the date the written consent was obtained. The authorized person obtaining the informed consent must also sign the ICF. A copy of the ICF(s) must be provided to the subject or the subject's legally authorized representative.

Subjects must be re-consented to the most current version of the ICF(s) during their participation in the study, as appropriate.

13.4. Financial Disclosure

The Principal Investigator and all sub-investigators of the study team will provide the Sponsor with sufficient, accurate financial information as requested to allow the sponsor to submit complete and accurate financial certification or disclosure statements to the appropriate regulatory authorities.

13.5. Insurance

The Sponsor will ensure that subjects are covered by relevant insurances covering subjects participating in the study. Liability for study induced injury will be covered according to local requirements.

14. REPORT AND PUBLICATION

Results from the study will be presented in a Clinical Study Report (CSR) as per ICH E3. The CSR will be signed by the sponsor and the Lead investigator in his role as Coordinating investigator for the study.

All information regarding RMC-035 supplied by the Sponsor to the Investigator or generated by the Investigator in accordance with the conduct of the study is privileged and confidential information of the Sponsor. The Investigator agrees to use this information to accomplish the study and will not use it for other purposes without written consent from the Sponsor. It is understood that there is an obligation to provide the Sponsor with complete data obtained during the study. The information obtained from the clinical study will be used by the Sponsor in connection with the development of RMC-035 and may be disclosed by the Sponsor to regulatory authorities, other Investigators, corporate partners, or consultants as required.

The Investigator's right and obligations with respect to publishing or otherwise presenting information regarding the study are detailed in the Publication provisions of the Clinical Study Agreement among the Investigator, the clinical site, and the Sponsor. The Investigator shall comply with such provisions.

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16. APPENDICES

Appendix 1: Contraceptive Guidance and Collection of Pregnancy Information

Appendix 2: Liver Laboratory Test Abnormalities

Appendix 3: Requisite Clinical Guidelines and Grading Scales

Appendix 4: Medical Outcomes Study (MOS) 36-Item Short Form Health Survey (SF-36)

Appendix 5: European Quality of Life 5 Domain 5-Level Score (EQ-5D-5L)

APPENDIX 1. CONTRACEPTIVE GUIDANCE AND COLLECTION OF PREGNANCY INFORMATION

Definitions:

Woman of Childbearing Potential (WOCBP)

A woman is considered fertile following menarche and until becoming post-menopausal unless permanently sterile (see below).

If fertility is unclear (eg, amenorrhea in adolescents or athletes) and a menstrual cycle cannot be confirmed before first dose of study intervention, additional evaluation should be considered.

Women in the following categories are not considered WOCBP

1. Premenarchal
2. Premenopausal female with 1 of the following:
   - Documented hysterectomy
   - Documented bilateral salpingectomy
   - Documented bilateral oophorectomy

   For individuals with permanent infertility due to an alternate medical cause other than the above, (eg, Mullerian agenesis, androgen insensitivity), investigator discretion should be applied to determining study entry.

   Note: documentation can come from the site personnel's review of the subject's medical records, medical examination, or medical history interview.
3. Postmenopausal female
   - A postmenopausal state is defined as no menses for 12 months without an alternative medical cause.
     - A high follicle stimulating hormone (FSH) level in the postmenopausal range may be used to confirm a postmenopausal state in women not using hormonal contraception or hormonal replacement therapy (HRT). However, in the absence of 12 months of amenorrhea, confirmation with more than one FSH measurement is required.
   - Females on HRT and whose menopausal status is in doubt will be required to use one of the non-estrogen hormonal highly effective contraception methods if they wish to continue their HRT during the study. Otherwise, they must discontinue HRT to allow confirmation of postmenopausal status before study enrollment.

Contraception Guidance:

Highly effective forms of birth control include:

- Combined (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation:
  - oral
  - intravaginal

  - transdermal
- Progestogen-only hormonal contraception associated with inhibition of ovulation:
  - oral
  - injectable
  - implantable
- Intrauterine device (IUD)
- Intrauterine hormone-releasing system (IUS)
- Bilateral tubal occlusion
- Vasectomized partner
- True sexual abstinence, defined as refraining from heterosexual intercourse during the entire period of risk associated with the IMP. The reliability of sexual abstinence needs to be evaluated in relation to the duration of the clinical trial and the preferred and usual lifestyle of the subject.

Collection of Pregnancy Information

Male subjects with partners who become pregnant

- The Investigator will attempt to collect pregnancy information on any male subject's female partner who becomes pregnant while the male subject is in this study. This applies only to male subjects who receive IMP.
- After obtaining the necessary signed informed consent from the pregnant female partner directly, the Investigator will record pregnancy information on the appropriate form and submit it to the sponsor within 24 hours of learning of the partner's pregnancy. The female partner will also be followed to determine the outcome of the pregnancy. Information on the status of the mother and child will be forwarded to the sponsor. Generally, the follow-up will be no longer than 6 to 8 weeks following the estimated delivery date. Any termination of the pregnancy will be reported regardless of fetal status (presence or absence of anomalies) or indication for the procedure.

Female Subjects who become pregnant

- The Investigator will collect pregnancy information on any female subject who becomes pregnant while participating in this study. The initial information will be recorded on the appropriate form and submitted to the sponsor within 24 hours of learning of a subject's pregnancy.
- The subject will be followed to determine the outcome of the pregnancy. The Investigator will collect follow-up information on the subject and the neonate and the information will be forwarded to the sponsor. Generally, follow-up will not be required for longer than 6 to 8 weeks beyond the estimated delivery date. Any termination of pregnancy will be reported, regardless of fetal status (presence or absence of anomalies) or indication for the procedure.

- While pregnancy itself is not considered to be an AE or SAE, any pregnancy complication or elective termination of a pregnancy for medical reasons will be reported as an AE or SAE.
- A spontaneous abortion (occurring at <22 weeks gestational age) or still birth (occurring at >22 weeks gestational age) is always considered to be an SAE and will be reported as such.
- Any post-study pregnancy related SAE considered reasonably related to the study intervention by the Investigator will be reported to the Sponsor as described in Section 9.7. While the investigator is not obligated to actively seek this information in former study subjects, he or she may learn of an SAE through spontaneous reporting.
- Any female subject who becomes pregnant while participating in the study will discontinue IMP administration.

APPENDIX 2. LIVER LABORATORY TEST ABNORMALITIES

Liver Safety Monitoring and Assessment

Any subject enrolled in this clinical study receiving IMP that reveals an increase of serum aminotransferases (AT) to >3 × Upper Limit of Normal (ULN), or total bilirubin (TBL) >2 × ULN, should undergo detailed testing for liver laboratory test abnormalities (including at least ALT, and TBL). Liver laboratory tests per protocol are performed at Screening, Baseline (before start of surgery), 24 hours, 48 hours and 72 hours after first IMP administration (see SOA, Table 3).

Testing should be repeated within 24 hours of notification of the initial test results in order to confirm the laboratory abnormality. Subjects should be asked if they have any symptoms suggestive of hepatobiliary dysfunction.

Definition of Liver Laboratory Test Abnormalities

Confirmed abnormalities will be characterized as moderate and severe:

Moderate >ALT 3× ULN or >TBL 2× ULN

Severe* >ALT 3× ULN and >TBL 2× ULN

In addition, the subject should be considered to have severe liver laboratory test abnormalities for any of the following:

- ALT or AST >8× ULN
- ALT or AST >5× ULN for more than 2 weeks
- ALT or AST >3× ULN and INR > 1.5 (If INR testing is applicable/evaluated).
- ALT or AST >3× ULN with the appearance of fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash and/or eosinophilia (> 5%).

The investigator may determine that abnormal liver laboratory test results, other than as described above, may qualify as moderate or severe abnormalities and require additional monitoring and follow-up.

*Hy's Law Definition-Drug-induced jaundice caused by hepatocellular injury, without a significant obstructive component, has a high rate of bad outcomes, from 10–50% mortality (or transplant). The two "requirements" for Hy's Law are:
1) Evidence that a drug can cause hepatocellular-type injury, generally shown by an increase in transaminase elevations higher 3 times the upper limit of normal ("2 x ULN elevations are too common in treated and untreated subjects to be discriminating")
2) Cases of increased bilirubin (at least 2 x ULN) with concurrent transaminase elevations at least 3x ULN and no evidence of intra- or extrahepatic bilirubin obstruction (elevated alkaline phosphatase) or Gilbert's syndrome.
[Temple R. Hy's law: predicting serious hepatotoxicity. *Pharmacoepidemiol Drug Saf* 2006 Apr;15(4):241-3.]

Follow-up Procedures

Confirmed moderate and severe abnormalities in liver laboratory tests should be thoroughly characterized by obtaining appropriate expert consultations, detailed pertinent history, physical examination and laboratory tests. The site should complete the Liver Abnormality Case Report Form (LA-CRF) that has been developed globally and can be activated for any study or an appropriate document.

Subjects with confirmed abnormal liver function testing should be followed up appropriately:

Confirmed moderate and severe liver laboratory test abnormalities should be repeated 2-3 times weekly then weekly or less until the abnormalities have normalized or considered stabilized. Severe liver laboratory test abnormalities as defined above, in the absence of another etiology, may be considered an important medical event and may be reported as a Serious AE (SAE). The Sponsor should be contacted and informed of all subjects for whom severe liver laboratory test abnormalities possibly attributable to IMP are observed.

To further assess abnormal liver laboratory test findings, the investigator is expected to:

Obtain a more detailed history of symptoms and prior or concurrent diseases. Symptoms and new onset-diseases should be recorded as 'AEs' on the AE page of the (e)CRF. Illnesses and conditions such as hypotensive events, and decompensated cardiac disease that may lead to secondary liver abnormalities should be noted. Non-alcoholic steatohepatitis (NASH) is seen in obese hyperlipoproteinemic, and/or diabetic subjects and may be associated with fluctuating aminotransferase levels. The investigator should ensure that the medical history form captures any illness that pre-dates study enrollment that may be relevant in assessing hepatic function.

Obtain a history of concomitant drug use (including non-prescription medication, complementary and alternative medications), alcohol use, recreational drug use, and special diets. Medications, including dose, should be entered on the concomitant medication page of the (e)CRF. Information on alcohol, other substance use, and diet should be entered on the LA-CRF or an appropriate document.

Obtain a history of exposure to environmental chemical agents. Based on the subject's history, other testing may be appropriate including:

- acute viral hepatitis (A, B, C, D, E or other infectious agents)
- ultrasound or other imaging to assess biliary tract disease
- other laboratory tests including INR, direct bilirubin

Consider gastroenterology or hepatology consultations.

Submit results for any additional testing and possible etiology on the LA-CRF or an appropriate document.

Investigational Medicinal Product Discontinuation

In the absence of an explanation for increased liver laboratory test abnormalities, such as viral hepatitis, pre-existing or acute liver disease or exposure to other agents associated with liver injury, the subject may be discontinued from the IMP. The investigator may determine that it is not in the subject's best interest to continue study treatment. IMP is to be discontinued in case of any of the following abnormal liver laboratory tests in blood (see also section 5.3.1):

- ALT >8 × ULN in any individual sample during the treatment period
- 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
- 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)
- ALT > 3 × ULN combined with total bilirubin > 2 × ULN in the same sample

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

Reference

Guidance for Industry titled "Drug-Induced Liver Injury: Premarketing Clinical Evaluation" issued by FDA on July 2009.

APPENDIX 3. REQUISITE CLINICAL GUIDELINES AND GRADING SCALES

KDIGO Guidelines for Grading Severity of Acute Kidney Injury

Stage	Serum Creatinine	Urine Output
1	1.5–1.9 times baseline OR ≥0.3 mg/dL (≥26.5 mmol/L) increase from baseline	<0.5 mL/kg/h for 6–12 hours
2	2.0–2.9 times baseline	<0.5 mL/kg/h for ≥12 hours
3	3.0 times baseline OR Increase in serum creatinine to ≥4.0 mg/dL (≥353.6 mmol/L) from baseline OR Initiation of RRT	<0.3 mL/kg/h for ≥24 hours OR Anuria for ≥12 hours

Abbreviation: RRT = renal replacement therapy.
Adapted from (Khwaja 2012)

New York Heart Association (NYHA) Functional Classification

NYHA Grading	
Class	Patient Symptoms
I	No limitation of physical activity. Ordinary physical activity does not cause undue fatigue, palpitation, dyspnea (shortness of breath).
II	Slight limitation of physical activity. Comfortable at rest. Ordinary physical activity results in fatigue, palpitation, dyspnea (shortness of breath).
III	Marked limitation of physical activity. Comfortable at rest. Less than ordinary activity causes fatigue, palpitation, or dyspnea.
IV	Unable to carry on any physical activity without discomfort. Symptoms of heart failure at rest. If any physical activity is undertaken, discomfort increases.

Adapted from (Dolgin 1994)

The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3) Guidelines

Adapted from (Singer 2016):

Sepsis is defined as life-threatening organ dysfunction caused by a dysregulated host response to infection. Organ dysfunction can be identified as an acute change in total Sequential (Sepsis-related) Organ Failure Assessment (SOFA) score ≥2 points consequent to the infection.

Sequential [Sepsis-Related] Organ Failure Assessment (SOFA) Score [a]

	Score				
System	**0**	**1**	**2**	**3**	**4**
Respiration					
PaO2/FIO2, mmHg (kPa)	≥400 (53.3)	<400 (53.3)	<300 (40)	<200 (26.7) with respiratory support	<100 (13.3) with respiratory support
Coagulation					
Platelets, $\times 10^3/\mu L$	≥150	<150	<100	<50	<20
Liver					
Bilirubin, mg/dL (µmol/L)	<1.2 (20)	1.2-1.9 (20-32)	2.0-5.9 (33-101)	6.0-11.9 (102-204)	>12.0 (204)
Cardiovascular	MAP ≥70 mm Hg	MAP <70 mm Hg	Dopamine <5 or dobutamine (any dose) [b]	Dopamine 5.1-15 or epinephrine ≤0.1 or norepinephrine ≤0.1 [b]	Dopamine >15 or epinephrine >0.1 or norepinephrine >0.1 [b]
Central nervous system					
Glasgow Coma Scale score [c]	15	13–14	10-12	6-9	<6
Renal					
Creatinine, mg/dL (µmol/L)	<1.2 (110)	1.2-1.9 (110-170)	2.0-3.4 (171-299)	3.5-4.9 (300-440)	>5.0 (440)
Urine output, mL/d				<500	<200

Abbreviations: FIO2, fraction of inspired oxygen; MAP, mean arterial pressure; PaO2, partial pressure of oxygen.
a. Adapted from Vincent et al.
b. Catecholamine doses are given as µg/kg/min for at least 1 hour.
c. Glasgow Coma Scale scores range from 3–15; higher score indicates better neurological function.

The baseline SOFA score can be assumed to be zero in patients not known to have preexisting organ dysfunction.

A SOFA score ≥2 reflects an overall mortality risk of approximately 10% in a general hospital population with suspected infection. Even patients presenting with modest dysfunction can deteriorate further, emphasizing the seriousness of this condition and the need for prompt and appropriate intervention, if not already being instituted.

In lay terms, sepsis is a life-threatening condition that arises when the body's response to an infection injures its own tissues and organs.

Patients with suspected infection who are likely to have a prolonged ICU stay or to die in the hospital can be promptly identified at the bedside with qSOFA, ie, alteration in mental status, systolic blood pressure ≥100 mm Hg, or respiratory rate ≥22/min.

Septic shock is a subset of sepsis in which underlying circulatory and cellular/metabolic abnormalities are profound enough to substantially increase mortality.

Patients with septic shock can be identified with a clinical construct of sepsis with persisting hypotension requiring vasopressors to maintain MAP ≥65 mm Hg and having a serum lactate level >2 mmol/L (18mg/dL) despite adequate volume resuscitation. With these criteria, hospital mortality is in excess of 40%.

APPENDIX 4. MEDICAL OUTCOMES STUDY (MOS) 36-ITEM SHORT FORM HEALTH SURVEY (SF-36)

Placehold

APPENDIX 5. EUROPEAN QUALITY OF LIFE 5 DOMAIN 5-LEVEL SCORE (EQ-5D-5L)

Placehold