CLINICAL STUDY PROTOCOL

Protocol Title:
A Phase 1b, Randomised, Double-Blind, Parallel Treatment Group Clinical Study to Evaluate the Safety, Tolerability and Pharmacokinetics of RMC-035 in Subjects Undergoing Non-Emergent On-Pump Coronary Artery Bypass Graft and/or Valve Surgery

Protocol Number: 20-ROS-04

Compound: RMC-035

Amendment Number: N/A

Study Phase: Phase 1b

Short Title:

A Phase 1b study of RMC-035 in subjects undergoing Cardiac Surgery

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Regulatory Agency Identifier Number(s)
EudraCT No.: 2020-004107-14

Approval Date:

22-DEC-2020

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1.1 Synopsis

Rationale:
This is the first clinical study in cardiac surgery patients and is being conducted to guide further clinical development in this patient population. Based on an integrated assessment of available nonclinical and clinical data, the number of doses and infusion time are considered to be safe 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.

Key Objectives and Key Endpoints:
Primary Objective:

- To evaluate the safety and tolerability of RMC-035 in subjects undergoing non-emergent, on-pump Coronary Artery Bypass Graft (CABG) and/or valve surgery

Secondary Objective:

- To evaluate the pharmacokinetics of RMC-035 in this patient population
- Identification and characterization of Anti-Drug-Antibodies (ADA) developed after multiple intravenous administrations of RMC-035

Primary Endpoint:

- Nature, frequency and severity of AEs

Secondary Endpoint:

- Clinically significant changes in safety laboratory tests (hematology, biochemistry, urinalysis)
- Clinically significant changes in vital signs (blood pressure, heart rate, respiratory rate)
- Clinically significant changes in 12-lead ECG
- Pharmacokinetics of RMC-035 in plasma: C_{max}, C_{trough}
- Pharmacokinetics of RMC-035 in plasma after **fourth** dose: AUCτ, C_{max}, C_{trough}, PTR, $R_{ac(AUC)}$, $t_{1/2}$
- Presence and titers of ADA 30 days after surgery
- Characteristics of ADA developed 30 days after surgery with regards to isotype, neutralizing capacity and cross-reactivity with endogenous alpha-1-microglobulin

Overall Design:

This is a study with two parallel treatment groups where subjects are randomised to receive RMC-035 or a matching placebo in a double-blind fashion.

Randomization will be performed on Day -1 where consented subjects, who are found eligible for study participation, will be randomised to receive intravenous dosing of either RMC-035 treatment or matching placebo in a 2:1 ratio.

Subjects must comply with the following key inclusion and exclusion criteria:

Key Inclusion Criteria:

- Female and male subjects with an age ≥18 years
- Subject is scheduled for non-emergent (elective) CABG and/or valve surgery (single or multiple valves) with use of cardiopulmonary bypass (CPB)
- Subject has at least ONE of the following risk factors for AKI at screening:
    - History of LVEF <35% for at least 3 months prior to screening assessed by either echocardiography, cardiac MRI or nuclear scan.
    - 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
    - Documented history of heart failure NYHA class II or higher for at least 3 months or longer at screening
    - Documented history of previous AKI before date of screening independent of the etiology of AKI
    - Documented history of anemia with hemoglobin ≤ 11 g/dL (≤6.8 mmol/L) for at least 3 months prior to screening
    - Documented history of albuminuria, defined as UACR >30 mg/g or > 30 mg/24 hour in a 24-hour urine collection.
    - eGFR is ≤ 60 mL/min/1.73 m^2 using the Chronic Kidney Disease – Epidemiology Collaboration (CKD-EPI) equation

Key Exclusion Criteria:

- Estimated glomerular filtration rate (eGFR) is <30 mL/min/1.73 m^2 using the Chronic Kidney Disease – Epidemiology Collaboration (CKD-EPI) equation at screening or at baseline
- Subject has surgery scheduled to be performed without CPB (“off-pump”)
- Subject has surgery scheduled for aortic dissection
- Subject is scheduled for CABG and/or valve surgery combined with additional non-emergent cardiac surgeries, e.g. for atrial fibrillation ablation
- Subject is scheduled to undergo trans catheter aortic valve implantation (TAVI) or trans catheter aortic valve replacement (TAVR), or single vessel off-pump surgeries or left ventricular device (LVAD) implantation
- 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) (Note: The prophylactic insertion of an IABP preoperatively for reasons not related to existing LV pump function is not exclusionary)

Disclosure Statement:

This is a randomised, parallel group treatment study with two (2) arms where subject and investigator are blinded.

Number of Subjects:
Twelve subjects undergoing elective on-pump CABG and/or valve surgery at high risk to develop peri- and post-surgery AKI will be enrolled in the study.
Two additional subjects may be enrolled at the Sponsor's discretion in case of early treatment discontinuations, which are not due to formal stopping criteria.

Treatment Groups and Duration:
The study will consist of three study periods:

- Screening: Day -1
- Treatment period: Day 1-3
- Safety follow-up period: Day 4-31.
  The follow-up period will consist of three visits (Day 4, Day 7 and Day 31).

A total of five doses of RMC-035 or placebo will be administered as IV infusions during the treatment period:

- First dose of RMC-035 is 1.3 mg/kg or placebo and will be administered over 60 minutes. Dose administration should start during surgery, 10 minutes before the expected start of cardiopulmonary bypass (CPB).
- Second, third, fourth and fifth doses of RMC-035 are 1.3 mg/kg per dose or placebo administration and will be administered over 30 minutes. Dose administrations start at 6, 12, 24 and 48 hours after the start of the first dose administration.
- Dose of RMC-035 will be reduced by 50% (i.e. 0.65 mg/kg per dose administration) in patients with eGFR 30 to <60 mL/min/1.73 m^2 at baseline.
- The dose of RMC-035 may be reduced for the 3rd, 4th and 5th IMP administration, depending on the renal function.

Data Monitoring Committee:
No data monitoring committee will be established and used in this clinical study.

1.2 Schedule of Assessments

Table 1 Schedule of Assessment – Complete Study Period

Assessments	Screening Period	Treatment Period			Follow-Up Period		
	1 day	IMP Administration			Up to 30 days after surgery		
Visit Number	1	2	3	4 (EOT)	5	6	7 (EOS) [e]
Visit Day	-1	1	2	3	4	7	31
Allowed visit window (days)	-5	±0	±0	±0	±0	±1	±3
Informed consent	x						
Medical history	x						
In/Exclusion criteria	x	x					
Demographics	x						
Weight and height [a]	x	x	x	x	x	x	x
Physical examination	x			x			
Prior and concomitant medication	x						
Serum pregnancy test (HCG) (WOCBP only)	x						x
Hematology lab	x	x	x	x	x	x	x
Clinical chemistry lab	x	x	x	x	x	x	x
Cardiac biomarkers (NT-proBNP, Troponin I)		x	x	x	x	x	x
Liver function lab	x	x	x	x	x	x	x
Serum Creatinine (SCr) and Cystatin C	x	x	x	x	x	x	x

Assessments	Screening Period	Treatment Period			Follow-Up Period		
	1 day	IMP Administration			Up to 30 days after surgery		
Visit Number	1	2	3	4 (EOT)	5	6	7 (EOS) [e]
Visit Day	-1	1	2	3	4	7	31
Allowed visit window (days)	-5	±0	±0	±0	±0	±1	±3
HbA1c	x						x
Urinalysis		x		x			
Randomization	x						
IMP administration		x	x	x			
Plasma PK sampling		x	x	x			
12-lead electrocardiogram	x	x	x	x		x	
Continuous ECG monitoring [b]		←	—	→			
Vital signs (BP, heart rate, respiratory rate)	x	x	x	x	x	x	
Surgery Assessments [c]		x					
Discharge from ICU [d]				←	—	→	
Hemodynamic Assessments (CO, SvO2, CVP)		x					
Urine output		←	—	→			
Sampling for biomarkers (plasma, serum and urine)		x	x	x	x		
Sampling for RMC-035 (urine)		x	x	x	x		
ADA assessment		x					x
AE and concomitant medication recording		←	—	—	—	—	→

[a] Height only measured at screening (visit 1)

[b] Cardiac surveillance from start of first infusion until 6 hours after start of last infusion

[c] Data being collected before, during and after surgery: time on CPB, duration of surgery, volume blood loss, administration of fluids (e.g. transfusion of red blood cells and osmotic agents) and time of admission to the ICU

[d] Time of discharge from ICU to hospital ward, another treatment facility or home

[e] Visit can take place either at the investigational site or at the patient's general practitioner

Table 2 Schedule of Assessment – Treatment Period

Visit Day	**Day 1**												**Day 2**	**Day 3**
Hours	**Pre-surgery [a]**	**0 [b]**	**0.25**	**0.5**	**1**	**2**	**3**	**4**	**5**	**6**	**8**	**12**	**24**	**48**
Visit Number	**2**												**3**	**4**
Inclusion/Exclusion criteria	X													
Concomitant medication [c]	X	←	—	—	—	—	—	—	—	—	—	→	x	x
Cardiac Surgery Assessments [d]		←	—	—	—	—	—	—	—	→				
IMP Administration		x [e]								x [f]		x [f]	x [f]	x [f]
Plasma PK sampling	X		x	x	x	x	x	x	x	x [g]		x [g]	x [g]	x [g]
12-lead electrocardiogram	X									x		x	x	x
Continuous ECG monitoring [h]														
Vital signs (BP, heart rate, respiratory rate)	X									x		x	x	x
Hematology lab	X									x		x	x	x
Clinical chemistry lab	X									x		x	x	x
Cardiac biomarkers (NT-proBNP, Troponin I)	X									x		x	x	x
Liver function tests	X												x	x
Serum Creatinine (SCr) and Cystatin C	X									x		x	x	x
Urinanalysis	X													x
Hemodynamic Assessments (CO, CVP) [l]	X						←	—	—	—	→		x	
Hemodynamic Assessments (SvO2)	X										x		x	
Sampling for biomarkers (plasma, serum and urine) [i]	X							x				x	x	x
Sampling for RMC-035 (urine)	X							x				x	x	x
Urinary output [k]	←	—	—	—	—	—	—	—	—	—	—	—	→	

Visit Day	**Day 1**												**Day 2**	**Day 3**
Hours	**Pre-surgery [a]**	**0 [b]**	**0.25**	**0.5**	**1**	**2**	**3**	**4**	**5**	**6**	**8**	**12**	**24**	**48**
Visit Number	**2**												**3**	**4**
Creatinine Clearance (CrCl) (urine) [m]												x	x	x
ADA assessment	X													
Adverse Events	←												→	

[a] Pre-surgery assessments will be performed within 60 minutes before surgery

[b] Time point 0 is defined as 10 minutes before expected onset of cardiopulmonary bypass (CPB)

[c] Including medications and anesthesia administered during surgery

[d] Collection before, during and after surgery: time on CPB, duration of surgery, volume blood loss, administration of fluids (e.g. transfusion of red blood cells and osmotic agents) and time of admission to the ICU.

[e] IV infusion over 60 minutes, first infusion should start 10 minutes before expected onset of CPB

[f] IV infusion over 30 minutes at 6 h, 12 h, 24 h and 48 h after the start of first infusion

[g] PK sampling will be performed at the following time points:
Day 1 (Infusion at 6 h): 6, 6.5, 7, 8 h
Day 1 (Infusion at 12 h): 12, 12.5, 13 (optional sample), 14 (optional sample) h
Day 2 (infusion at 24 h): 24, 24.5, 25, 26. 27, 28, 29 h
Day 3 (infusion at 48 h): 48, 48.5, 49, 50 h.

[h] Cardiac surveillance from start of first infusion until 6 hours after start of last infusion

[i] Detailed description on analytes at each specific time point is described in Table 5

[k] Urinary output will be measured at an hourly basis for 48 h according to site standard procedure

[l] Cardiac output (CO) will be measured pre-surgery, immediately after surgery and at 24 h via TTE (transthoracic echocardiography) or TEE (transesophageal echocardiography). Central venous pressure (CVP) will also be measured pre-surgery, immediately after surgery as well as at 24 h.

[m] CrCl will be calculated in collected urine between IMP administrations, starting before the 3rd IMP administration.
Day 1 (IMP infusion at 12 h): urine collection interval 6-10 h
Day 2 (IMP infusion at 24 h): urine collection interval 18-22 h
Day 3 (IMP infusion at 48 h): urine collection interval 42-46 h (OR eGFR will be calculated using SCr (CKD-EPI), if the urine collection via urine catheter has been stopped at this time point)

2 INTRODUCTION

The investigational medicinal product (IMP), RMC-035, contains the pharmacologically active protein RMC-035, a recombinant variant of endogenous human alpha-1-microglobulin (A1M).

RMC-035 is being developed for the prevention and treatment of AKI in patients undergoing cardiac surgery. 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, progression to chronic kidney disease (CKD) and mortality. Thus, a single AKI episode after cardiac surgery can lead to a significant burden of comorbidities, prolonged hospital stays, poor quality of life and high long-term costs.

RMC-035 has been evaluated in three phase 1 clinical studies: 17-ROS-01 (single ascending dose study); 19-ROS-02 (multiple ascending dose study); and 19-ROS-03 (renal impairment study). Based on available clinical and non-clinical data, RMC-035 is assessed as safe and generally well tolerated both in healthy subjects and in subject with renal impairment.

2.1 Study Rationale

This is a phase 1b, randomised, double-blind, parallel treatment group clinical study, to assess safety, tolerability and pharmacokinetics of RMC-035 in subjects undergoing non-emergent on-pump coronary artery bypass graft and/or valve surgery. This is the first clinical study in cardiac surgery patients and is being conducted 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 safe 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.

2.2 Background

2.2.1 Pharmacology

The investigational medicinal product (IMP) RMC-035 is a recombinant variant of the endogenous human protein alpha-1-microglobulin (A1M). RMC-035 is a potent antioxidant, harbouring 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 [1] derives from animal models of ischemic AKI. In addition, a number of other animal models related to renal injuries, e.g. 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.

2.2.2 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 have been 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. Immunohistochemical 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).

No observed adverse effect levels (NOAEL) were established in all GLP-studies and have been set at 25/20 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 (AUC).

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) indicating that higher exposure levels would be safe. A conservative approach has been adopted that has used the 28-day study exposure levels to define the maximum exposure levels in humans.

2.2.3 Summary of Clinical Studies

2.2.3.1 Single Ascending Dose Study (17-ROS-01)

In study 17-ROS-01 (SAD), five dose levels were evaluated 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 AEs, vital signs, 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 RMC-035 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 h after dosing in the highest dose group. For more details, please refer to the Investigator's Brochure (IB).

2.2.3.2 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, respectively. Six subjects were included per dose group to receive either RMC-035 or placebo (4:2 randomization) as multiple doses over 48 h. 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 h 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. ISRs were characterized by varying symptomatology

including localized redness, tenderness, pain and swelling. In one subject an ISR led to early discontinuation after the 5th administration. 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. In this study the use of an anti-coagulant ointment resulted in a shorter duration of symptoms.
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 Leucocyte count was observed in higher dose groups at the 12-hour time point, which was considered not clinically significant by the investigator. Furthermore, apparent reductions in serum creatinine, 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.

2.2.3.3 Renal Impairment Study (19-ROS-03)

Study 19-ROS-03 is an ongoing 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 eGFR ≥15 and < 90 mL/min/1.73 m^2 not on dialysis is planned to be included and allocated into one of five estimated GFR (eGFR) strata based on their renal function. Distribution of subjects were as follows: 1 subject in stratum 1 (≥75-<90), 1 subject in stratum 2 (≥60-<75) and 2 subjects per stratum in strata 3 to 5, i.e. ≥45-<60, ≥30-<45 and ≥15-<30, respectively.

Complete safety data and pharmacokinetic results are available for subjects in strata 1 to 4 who received a single IV infusion of RMC-035 over 30 minutes at a dose of 0.43 mg/kg. Results are to be considered preliminary since database lock did not yet occur.

RMC-035 was safe and well tolerated based on AEs, vital signs, ECGs, physical examinations and safety laboratory parameters. There were no deaths or other serious adverse events (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 study drug and resolved spontaneously approximately 2 hours after onset.

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

2.2.3.4 Anti-Drug-Antibodies (ADA)

Three subjects in the SAD study (17-ROS-01) and ten subjects in the MAD study (19-ROS-02) developed confirmatory titers of ADA during follow-up at 20-22 days after dosing.
In the renal impairment study (19-ROS-03), one of eight subjects had confirmatory titers of ADA at follow-up.

For a more detailed overview of the safety and pharmacology of RMC-035, please refer to the Investigator's Brochure (IB) [1].

2.3 Benefit/Risk Assessment

More detailed information about the known and expected benefits and risks and reasonably expected adverse events of RMC-035 may be found in the Investigator's Brochure [1].

2.3.1 Key Safety Information

Preclinical experience includes an extensive safety toxicology program with Good Laboratory Practice (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 toxicity 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}). These studies confirm that potential renal toxicity is primarily related to the maximum exposure (C_{max}) and not cumulative exposure (AUC). The maximum C_{max} levels in the SAD study cohort with 2.6 mg/kg was 38.8 µg/mL whilst the highest C_{max} levels in the MAD study cohort with 1.3 mg/kg administered 6 times over 48 hours was 19.5 µg/mL. These levels are still below the NOAEL levels of 60 µg/mL (marmoset 28-day study) and 161 µg/mL (cyno 3-day study) in the relevant toxicology studies. RMC-035 is rapidly distributed to the kidney, with a terminal half-life ($t_{1/2}$) of around 4 h in marmoset, 5 h in cynomolgus monkey and a human $t_{1/2}$ of 16 h in the MAD study.

RMC-035 was evaluated in 42 subjects in three clinical studies in healthy subjects and subjects with varying degrees of renal function. The most reported adverse events were ISRs, headache, nausea and vomiting. Anti-Drug Antibodies were confirmed in the majority of subjects who received multiple doses of RMC-035.

The following risk mitigation factors are applied for this study:

ISRs:

- IMP will be administered in a central venous line whenever possible (use of central venous catheters is considered standard of care in cardiac surgery)
- IMP will be diluted in a sodium chloride infusion buffer prior to administration and will be administered with higher infusion rate to minimize local irritancy
- 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, e.g. with topical anti-coagulants and cooling.

Renal protein overload attributable to high RMC-035 C_{max}:

- Maximum clinical dose is set to 1.3 mg/kg per dose administration. The risk for renal protein overload with this dose is considered small. No signs of renal tubular injury, tubular stress or changes in renal function were observed so far in clinical studies at higher exposure levels, and no relevant accumulation (i.e. increase in C_{max}) is expected based on the short treatment duration and rapid initial elimination of RMC-035 from plasma (approximately 70-80% of RMC-035 is eliminated from plasma within one hour after end-of-infusion).
- As a precautionary measure for this first clinical study in cardiac surgery patients, subjects with eGFR<60 mL/min/1.73 m^2 will have the administered dose reduced 0.65 mg/mg as modeling of PK data in renally impaired subjects demonstrated an approximately doubling of RMC-035 exposure (AUC) at this eGFR level.
- Exclusion of subjects with baseline eGFR<30 mL/min/1.73 m^2

- Due to possible changes in renal function during the study the dose for the 3rd, 4th and 5th IMP administration will be adjusted based on actual renal function as defined in section 6.6.
- Development of AKI stage 3 as per KDIGO criteria including requirement for renal replacement therapy (i.e. dialysis) is a formal stopping criterion (see Section 7.2).

Development of ADAs:

- The potential risks associated with ADAs are limited given the short treatment duration (<3 days) and rapid initial plasma clearance of RMC-035. Thus, potential formation of ADAs are not expected to have impact on 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.

2.3.1.1 Risks associated with the Patient Population and Study Assessments

- Subjects undergoing cardiac surgery represent a high-risk population for developing cardiovascular events, e.g. cardiac ischemia and arrhythmias. Subjects are also expected to be at increased risk to develop a post-operative decline in renal function due to the surgical procedure.

2.3.1.2 General and Standard Precautions in the study

- Close monitoring of Subjects during and after IMP administration. Subjects will remain at the Investigational site (i.e. ICU or post-operative ward) immediately following cardiac surgery and will be closely monitored by medical staff during the full treatment period.
- The Investigational Site will ascertain that adequate facilities and procedures are available to handle emergency situations should they occur during the study.
- Each subject will be provided with a subject information card with information about the study, the IMP, subject study ID, name of the Investigator and an emergency number.

2.3.2 Benefit Assessment

- This is the first study of RMC-035 in cardiac surgery, and the benefit of RMC-035 for prevention and treatment of AKI is currently unknown.
- For subjects allocated to receive placebo, no benefit can be expected.

2.3.3 Overall Benefit: Risk Assessment

Overall, a careful risk mitigation strategy as outlined above has been implemented based on non-clinical and clinical experience with RMC-035.

Taking this into account, the potential risks identified in association with RMC-035 are justified and well-balanced given the study objectives and information gain to guide further clinical development.

3 OBJECTIVES AND ENDPOINTS

3.1 Primary, secondary and exploratory objectives

3.1.1 Primary objective:

- To evaluate the safety and tolerability of RMC-035 in subjects undergoing non-emergent, on-pump Coronary Artery Bypass Graft (CABG) and/or valve surgery

3.1.2 Secondary objective:

- To evaluate the pharmacokinetics of RMC-035 in this patient population
- Identification and characterization of ADA developed after multiple intravenous administrations of RMC-035

3.1.3 Exploratory objectives:

- To evaluate post-baseline changes in biomarkers of renal function and tubular damage/stress
- To evaluate post-baseline changes in biomarkers of oxidative stress and inflammatory cytokines
- To evaluate post-baseline changes in markers of cardiovascular and hemodynamic function

3.2 Primary, secondary and exploratory endpoints

3.2.1 Primary endpoint

- Nature, frequency and severity of AEs

3.2.2 Secondary endpoint

- Clinically significant changes in safety laboratory tests (hematology, biochemistry, urinalysis)
- Clinically significant changes in vital signs (blood pressure, heart rate, respiratory rate)
- Clinically significant changes in 12-lead ECG
- Pharmacokinetics of RMC-035 in plasma: C_{max}, C_{trough}
- Pharmacokinetics of RMC-035 in plasma after **fourth** dose: AUCτ, C_{max}, C_{trough}, PTR, $R_{ac(AUC)}$, $t_{1/2}$
- Presence and titers of ADA 30 days after surgery
- Characteristics of ADA developed 30 days after surgery with regards to isotype, neutralizing capacity and cross-reactivity with endogenous alpha-1-microglobulin

3.2.3 Exploratory endpoint(s)

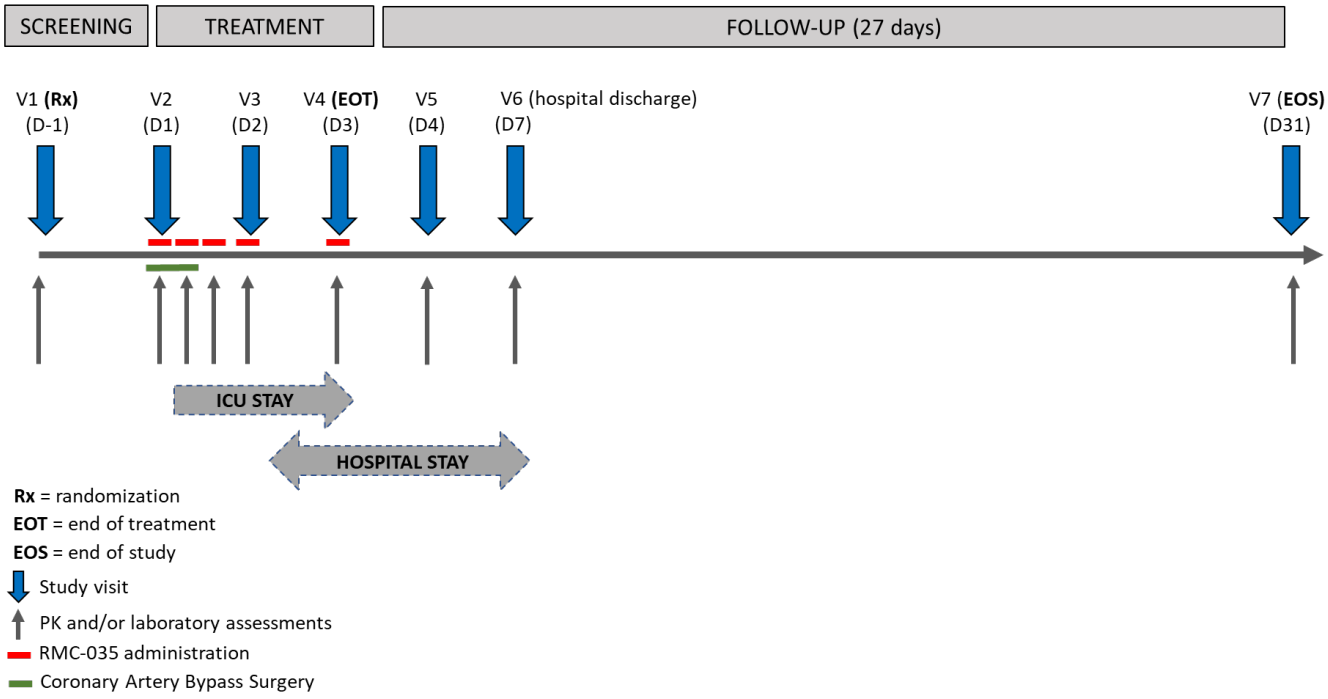
- Post-baseline changes in specific categories of biomarkers:
  - renal tubular damage and stress markers, e.g.
    KIM-1, TIMP2, IGFBP7, IL-18, L-FAPB, NGAL (urine)
    KIM-1, NGAL and A1M (plasma)
  - oxidative stress markers e.g. 8OH-dG (serum and urine)
  - inflammatory cytokines e.g. IL-6 and IL-8 (plasma)
  - cardiac biomarkers, e.g. NT-proBNP and Troponin I
- Concentration of RMC-035 in urine
- Post-baseline changes in hemodynamic assessments, including cardiac output (CO), mixed venous saturation (SvO2) and central venous pressure (CVP).
- Presence of major adverse kidney event at 30 days (MAKE30), defined as a composite
  of death, dialysis or ≥ 25% eGFR reduction based on either CKD-EPI or modified CKD-EPI equations, respectively.
- Percent increase and maximum percent increase in SCr and Cystatin C (and corresponding estimated GFR values based on CKD-EPI and modified CKD-EPI equations, respectively) from baseline
- Post-baseline AUC of Serum Creatinine (SCr) and Cystatin C levels (and corresponding eGFR values) from baseline to 48 hours, 72 hours and 7 days post-surgery
- Severity of AKI defined as the following:
  - Stage 1: SCr 1.5 to 1.9 times baselinewithin 7 days, OR ≥0.3 mg/dL (≥ 26.5 µmol/L) within 48 hours OR urine output <0.5 mL/min/h for 6 to <12 hours
  - Stage 2: SCr 2.0-2.9 times baseline within 7 days OR urine output <0.5 mL/min/h for ≥ 12 hours
  - Stage 3: SCr 3.0 times baseline within 7 days, OR increase in SCr 5.0 mg/dL (≥353.6 µmol/L), OR initiation of renal replacement therapy OR urine output <0.3 mL/min/h for ≥24 hours OR anuria for ≥12 hours
- Duration and persistence of AKI defined as either the following
  - The number of days from start of AKI (KDIGO definition) [5] where either SCr increase ≥ 0.3 mg/dL above pre-AKI reference point (first comparison is versus pre-surgery baseline, or a reference value after baseline if AKI occurs after 48 hours post-surgery) or if SCr increase is ≥ 1.5 times baseline or dialysis in the first 7 days, or up to discharge if prior to 7 days
  - Persistent AKI defined as AKI (KDIGO definition) for >48 hours
- Length of index Intensive Care Unit (ICU) stay and index ICU 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 admission to hospital discharge for the index surgery
- Nature of patient discharge facility (e.g. home, skilled nursing facility, rehabilitation center)

4 STUDY DESIGN

4.1 Overall Design

- This is a phase 1b, randomised, double-blind, parallel treatment group clinical study, to assess safety, tolerability and pharmacokinetics of RMC-035 in subjects undergoing non-emergent on-pump coronary artery bypass graft and/or valve surgery.
- Twelve (12) subjects will be enrolled at a single investigational site and randomised to receive either RMC-35 or matching placebo in a 2:1 ratio. Two (2) additional subjects may be enrolled at the Sponsor's discretion in case of early treatment discontinuations, which are not due to formal stopping criteria. The study will consist of three study periods:
    - Screening (Visit 1 on Day -1(-5 days)),
    - 3-days treatment period (Visit 2-4 on Day 1-3)
    - Up to 30 days safety follow-up period (Day 4-31).
      The follow-up period will consist of three visits (Visit 5-7) at Day 4, Day 7 (±1day) and Day 31 (±3 days). Visit 7 can take place either at the investigational site or if not possible at the subjects general practitioner after agreement with the Investigator.
- Randomization will be performed on Day -1 where consented subjects, who are found eligible for study participation, will be randomised to receive IV dosing of either RMC-035 treatment or matching placebo:
- In total five (5) doses of RMC-035 will be administered as follows:
    - First dose is 1.3 mg/kg and administered over 60 minutes. Dose administration should start during surgery, 10 minutes before the expected start of cardiopulmonary bypass (CPB).
    - Second, third, fourth and fifth dose is 1.3 mg/kg per dose administration and administered over 30 minutes. Dose administrations start at 6, 12, 24 and 48 hours after the start of the first dose administration.
    - Placebo will be dosed at identical time points and infusion rates as RMC-035.
- Assessment (type and time points) during screening, treatment period and safety follow-up period are detailed in the Schedule of Assessment (Section 1.2).

Figure 1 Study Flowchart



4.2 Scientific Rationale for Study Design

RMC-035 is under clinical development for the prevention and treatment of AKI in patients undergoing cardiac surgery (CABG and/or valve replacement) with the use of CPB. This is the first study of RMC-035 in cardiac surgery patients and will provide an initial assessment of the safety, tolerability and pharmacokinetic properties in the target population for future clinical development. An early exploratory assessment of efficacy will be performed by analysing panels of biomarkers and post-operative changes in renal function.

A placebo control will be used to establish the frequency and magnitude of changes in endpoints that may occur in the absence of active treatment. Treatment allocation is randomised to minimize bias in the assignment of subjects to treatments. Blinded treatment allocation will reduce potential bias during data collection and evaluation of endpoints.

4.3 Dose and Dose regimen

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 (MAD study). In this Phase 1b safety and tolerability study in cardiac surgery patients the following dosing regimen is applied:

- RMC-035 will be administered 5 times in 48 hours
- The first dose is 1.3 mg/kg administered over a one hour infusion
- RMC-035 will then be administered in a dose of 1.3 mg/kg during 30 minutes at 6, 12, 24 and 48 hours after the start of the first administration
- For subjects with an eGFR at baseline of < 60mL/min/1.73m^2 the dose will be reduced to 0.65 mg/kg

- The dose of RMC-035 for the 3rd, 4th and 5th administration may be reduced depending on the renal function (see Section 6.6). RMC-035 will be diluted and administered in a fixed volume 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 after surgery, IMP may be administered via a catheter in a peripheral vein. This catheter should preferably be reserved for IMP administration only.

This dosing regimen is considered to provide intra- and post-operative renal protection of RMC-035 considering the PK and non-clinical pharmacology data.

4.3.1 Dose Adjustment for Renal Function at baseline

Preliminary results of the study in subjects with renal dysfunction (19-ROS-03) show that elimination of RMC-035 is dependent on renal function. As a precautionary measure for the first clinical study in cardiac surgery patients, subjects with a GFR<60 mL/min/1.73 m^2 will have the administered dose reduced to 0.65 mg/kg as modeling of the 19-ROS-03 renal impaired subjects demonstrated a doubling of RMC-035 exposure (AUC).

4.4 End of Study Definition

A subject is considered to have completed the study if he/she has completed the full study period including end of study visit 7 on Day 31 ±3 days. All randomised subjects will be followed up to Day 31 for AEs and attainment of predefined study endpoints.

The end of the study is defined as the date when the last visit of the last subject in the study at the Investigational site has occurred.

5 STUDY POPULATION

Twelve (12) subjects undergoing elective on-pump CABG and/or valve surgery with risk factors to develop peri- and post-surgery AKI will be enrolled in the study.

Two additional subjects may be enrolled at the Sponsor's discretion in case of early treatment discontinuations which are not due to formal stopping criteria.Prospective approval of protocol deviations to enrollment criteria, also known as protocol waivers or exemptions, will NOT be allowed.

5.1 Inclusion Criteria

Subjects are eligible to be included in the study only if all of the following criteria apply:

1. 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 has provided written informed consent
3. Subject age is ≥18 years
4. Subject is scheduled for non-emergent (elective) CABG and/or valve surgery (single or multiple valves) with use of cardiopulmonary bypass (CPB)
5. Subject has at least ONE of the following risk factors for AKI at screening:
    a. History of LVEF <35% for at least 3 months prior to screening assessed by either echocardiography, cardiac MRI or nuclear scan.
    b. History of previous open chest cavity cardiac surgery with or without CPB
    c. Confirmed diagnosis of type 2 diabetes (T2DM) at least 3 months prior to screening AND ongoing treatment with an approved anti-diabetic drug
    d. Age ≥70 years
    e. Documented history of heart failure NYHA class II or higher for at least 3 months or longer at screening
    f. Documented history of previous AKI before date of screening independent of the etiology of AKI
    g. Documented history of anemia with hemoglobin ≤ 11 g/dL (≤6.8 mmol/L) for at least 3 months prior to screening
    h. Documented history of albuminuria, defined as UACR >30 mg/g or > 30 mg/24 hour in a 24-hour urine collection.
    i. eGFR is ≤ 60 mL/min/1.73 m^2 using the Chronic Kidney Disease – Epidemiology Collaboration (CKD-EPI) equation
6. Female subject is either:
    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)

    Or if of childbearing potential
        - agree not to try to become pregnant during the study and for 28 days after the final study drug administration
        - must have a negative serum pregnancy text at screening and
        - if heterosexually active, agree to consistently use a highly effective form of birth control* starting at screening and throughout the study period, and continue to do so for 28 days after final study treatment administration. If using hormonal contraception this method must be supplemented with a barrier method, preferably male condom.
7. Female subject must not be breastfeeding at screening or throughout the study period and for 28 days after the final study treatment administration

8. Female subject must not donate ova/egg starting at screening and throughout the study period and for 28 days after final study drug administration
9. Male subject and their female spouse/partner(s) who are of childbearing potential must be using a highly effective form of birth control starting at screening and continue to do so throughout the study period and for 12 weeks after final study treatment administration.
10. Male subjects must not donate sperm starting from screening, throughout the study period and up to 12 weeks after final study drug administration
11. Subject agrees not to participate in another interventional study from the time of signing the informed consent until the End of Study visit (EOS)

*Highly effective forms of birth control include [3]:

- 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
- Vasectomised partner
- True sexual abstinence, defined as refraining from heterosexual intercourse during the entire period of risk associated with the study treatments. 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.

5.2 Exclusion Criteria

Subjects will be excluded from participation in the study if any of the following criteria apply:

1. Subject has any medical condition that in the opinion of the investigator makes the subject unsuitable for study participation
2. Estimated glomerular filtration rate (eGFR) is <30 mL/min/1.73 m^2 using the Chronic Kidney Disease – Epidemiology Collaboration (CKD-EPI) equation at screening or at baseline
3. Subject has surgery scheduled to be performed without CPB (“off-pump”)
4. Subject has surgery scheduled for aortic dissection
5. Subject has surgery to correct any major congenital heart defect
6. Subject is scheduled for a condition that is immediately life-threatening
7. Subject is scheduled for CABG and/or valve surgery combined with additional non-emergent cardiac surgeries, e.g. for atrial fibrillation ablation
8. Subject is scheduled to undergo trans catheter aortic valve implantation (TAVI) or trans catheter aortic valve replacement (TAVR), or single vessel off-pump surgeries or left ventricular device (LVAD) implantation
9. Subject experiences a cardiogenic chock 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 or anticipated to be required during surgery
10. 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) (Note: The prophylactic insertion of an IABP preoperatively for reasons not related to existing LV pump function is not exclusionary)

11. Required cardiopulmonary resuscitation within 14 days prior to cardiac surgery
12. Ongoing sepsis (as defined by SEPSIS-3, the Third International Consensus Definitions for Sepsis and Septic Shock) or history of sepsis within the past 2 weeks or untreated diagnosed infection prior to or at screening and before randomization.
13. Total bilirubin or alanine aminotransferase (ALT) or aspartate aminotransferase (AST)≥ 2 times the upper limit of normal (ULN) at screening
14. Subject has a history of kidney transplantation
15. Subject has received an investigational medicinal product in the last 90 days (or after 5 half-lives of the investigational drug, whichever is longer
16. Subject has a known allergy to, RMC-035 or one if its excipients, or has previously received, RMC-035

5.3 Screen Failures

Screen failures are defined as subjects who consent to participate in the clinical study but are not eligible for participation according to inclusion/exclusion criteria. A minimal set of screen failure information is required to ensure transparent reporting of screen failure subjects to meet the Consolidated Standards of Reporting Studies (CONSORT) publishing requirements and to respond to queries from regulatory authorities.
Minimal information includes informed consent date, demography, reason for screen failure details and any serious adverse event (SAE).

Individuals who do not meet the criteria for participation in this study (screen failure) may not be re-screened. If the subject has failed one of the inclusion criteria or fulfilled one of the exclusion criteria related to laboratory parameters, re-sampling is not allowed.
However, in case of technical issues (e.g. haemolysed or lost), re-sampling is allowed for the affected parameters but this is not regarded as re-screening.

6 TREATMENTS

Study treatment is defined as investigational medicinal product (IMP) including placebo intended to be administered to a study subject according to the study protocol.

6.1 Treatments(s) administered

The IMPs in this study are RMC-035 and its corresponding Placebo.
RMC-035 contains a pharmacological active protein RMC-035 which is a recombinant version of the endogenous human protein alpha-1-microglobulin.

RMC-035 and Placebo are manufactured according to Good Manufacturing Practice (GMP) standards for this study on behalf of the sponsor. Instructions for safe receipt, handling and storage of RMC-035 are described in the IMP Manual.

Table 3 IMP characteristics

IMP 1	
Compound Name	RMC-035
Pharmaceutical form	Solution for injection
Strength	4.0 mg/mL (10 mL vial containing 40 mg in total)
Route of administration	Intravenous infusion
Dose	First dose 1.30 mg/kg – infusion over 60 minutes. Remaining dose administrations at 6, 12, 24 and 48 hours after the start of the first dose – infusion over 30 minutes
Physical characteristics	Sterile, clear, particle free and colourless or slightly yellow liquid
Excipients	Sodium phosphate 10 mM pH 7,4, NaCl 0.15 M, L-Histidine 2 mg/mL
Packaging	10 mL glass vials sealed with 20 mm serum stoppers, crimped with caps
Storage	≤ - 70°C
IMP 2	
Compound Name	Placebo for RMC-035
Pharmaceutical form	Solution for injection
Strength	N/A
Route of administration	Intravenous infusion
Dose	Placebo with identical infusion times and duration as RMC-035
Physical characteristics	Sterile, clear, particle free and colourless or slightly yellow liquid
Excipients	Sodium phosphate 10 mM pH 7,4, NaCl 0.15 M, L-Histidine 2 mg/mL
Packaging	10 mL glass vials sealed with 20 mm serum stoppers, crimped with caps
Storage	-20 ±5°C

6.2 Preparation/Handling/Storage/Accountability

6.2.1 Packaging and labelling of IMP

Packaging and labelling will be performed in accordance with GMP and national regulatory requirements [2].

RMC-035 solution for injection and its corresponding Placebo are filled in glass vials each containing an extractable volume of 10 mL solution. The vials will be packaged in a suitable carton box, each containing 8 vials.

RMC-035 solution for injection 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.

6.2.2 Storage of IMP

Vials with RMC-035 must be stored at ≤ - 70°C and vials with Placebo at -20 ±5°C 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 the storage within the right 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.

6.2.3 Preparation of IMP

Preparation of the IMP will be done by trained staff at the investigational site in a dedicated area. There will be two unblinded persons working together, one performing the preparation and one supervising the process.

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 patient.

The diluted product may be stored in a prefilled infusion syringe in a refrigerator (+2-8°C) for a maximum of 72 hours. Administration to the subject shall be completed within 2 hours after the syringe has been taken out of the refrigerator.

Detailed guidance for preparation of the IMP including which auxiliary supplies to use during preparation will be described in a separate IMP Manual or equivalent.

6.2.4 Administration of IMP

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

RMC-035/Placebo 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 investigational site.
The IMP should be administered via a central venous catheter (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, around 10 minutes before the anticipated initiation of CPB. The first infusion (1.3 mg/kg or 0.65 mg/kg) will be administered IV at an infusion rate of 0.8 mL/min. Start time of the first dose administration will serve as reference point for further dose administrations and protocol-mandated assessments.

Four additional doses will be given as IV infusions (1.3 mg/kg/dose or 0.65 mg/kg/dose), at an infusion rate of 1.6 mL/min, 6, 12, 24 and 48 hours respectively after start of the first infusion. The volume to be infused over 30 minutes will be 33 mL and the infusion rate 1.1 mL/min in case the dose is reduced to 0.43 mg/kg/dose in any of the IMP administrations 3-5, as described in section 6.6.
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.

Detailed guidance for administration of the IMP including which syringe pump and auxiliary supplies to use will be described in a separate IMP Manual or equivalent.

6.2.5 Accountability of IMP

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.

6.2.6 Return and Destruction of IMP

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 and will be done according to local procedures after accountability is finalised by the site and reconciled by the monitor. Documentation on destruction will be forwarded to the sponsor.

Any unused IMP will be returned for destruction to the sponsor or sponsor representative after drug accountability has been performed and verified by the monitor.

6.3 Measures to Minimize Bias; Randomization and Blinding

This is a double-blind study and the allocation of treatments will not be disclosed until after declaration of clean file and the database has been locked.

Eligible patients will on Day -1 subjects be assigned a unique number (randomization number) in ascending numerical order at the investigational site. The randomization number encodes the subject's assignment to one of two arms of the study, according to the randomization schedule generated prior to the study by the Statistics Department at KLIFO. Each subject will be dispensed blinded IMP, at the same dose, labelled with his/her unique randomization number, throughout the study.

A sealed envelope that contains the treatment allocation for each subject will be provided to the investigator. The sealed envelope will be retained by the investigator (or representative) in a secured area with restricted access. In case of an emergency, the investigator has the sole responsibility for determining if unblinding of a subjects' IMP dose is warranted. Subject safety must always be the first consideration in making such a determination. If the

investigator decides that unblinding is warranted, the investigator should make every effort to contact the sponsor prior to unblinding a subject's IMP dose unless this could delay emergency treatment of the subject. If a subject's IMP dose is unblinded, the sponsor must be notified within 24 hours after breaking the blind. Once the study is complete, all envelopes (sealed and opened) must be accounted for and returned to the sponsor.

A similar set of decoding envelopes will be maintained at KLIFO PV, in a secured area with restricted access.

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 eCRF.

The dose of IMP and study subject identification will be confirmed at the time of dosing by a member of the investigational site staff other than the person administering the study product.

6.5 Concomitant Therapy

Any prescription and non-prescription medications (e.g. 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 and frequency

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

Changes, additions, or discontinuations to medications will be assessed and recorded in the eCRF during each study visit. If a change is due to an AE, then this must be reported according to Appendix 3. The Sponsor should be contacted if there are any questions regarding concomitant or prior therapy.

6.5.1 Restricted concomitant medications:

- Antioxidant supplementation (e.g. vitamin C and E) should be discontinued before randomization and up to patient discharge
- Nephrotoxins (e.g. metformin, NSAIDs) should be discontinued before randomization and up to patient discharge
- Angiotensin Converting Enzyme inhibitors (ACEi) or Angiotensin Receptor Blockers (ARB) should be discontinued on the day of hospital admission and preferably avoided until 72 hours after start of surgery. ACEi/ARB may be used within 72 hours after surgery if clinically indicated.

6.6 Dose Modification

The dose of RMC-035 may be reduced for IMP administrations 3, 4 and 5 based on renal function. Creatinine Clearance (CrCl as mL/min) will be measured before the 3rd, 4th and 5th IMP administration to decide on possible dose reduction. If the urine catheter is removed after 24 hours the dose for the 5th IMP administration at 48 hours will be based on the eGFR (as mL/min/1.73m^2) based on serum creatinine as per the CKD-EPI equation.

- For the 3rd and 4th IMP administration CrCl will be used to estimate renal function
- For the 5th IMP administration CrCl OR eGFR will be used to assess renal function

The renal function will determine the dose:

- Patients with a CrCl/eGFR <60 mL/min will receive 0.65 mg/kg per dose administration
- Patients with a CrCl/eGFR <30 mL/min will receive 0.43 mg/kg per dose administration

The dose of IMP will not be increased once a decrease has been made.

6.7 Treatment after the End of the Study

When discontinuing the IMP, the subject should be transferred to local standard of care if needed at the discretion of the investigator or treating physician.

7 TEMPORARY STUDY HALT, DISCONTINUATION OF IMP AND SUBJECT DISCONTINUATION/WITHDRAWAL

7.1 Temporary Study Halt

- If one grade 4 Adverse Event is reported, which is an Injection Site Reaction (ISR) or Infusion Related Reaction (IRR) and which is considered to be an immune mediated reaction and possibly related to IMP, recruitment of new subjects into the study will be halted temporarily. Dosing of ongoing subjects will also be discontinued. All subjects will remain in the study and continue follow up until scheduled end of study.
- If a grade 3 Adverse Event is reported in ≥2 subjects, which is an ISR or IRR which are considered immune mediated reactions and possibly related to IMP, further recruitment will be stopped. Subjects who are ongoing will continue dosing of IMP unless any ISR or IRR related event of any grade is reported in these subjects. All subjects will remain in the study and continue follow up until scheduled end of study.

If a Temporary Study Halt criterion is met as described above, the Sponsors Medical Representative and the Principal Investigator will assess the individual case(s) and report the assessment to the competent authority and Ethics Committee.

The study will only continue recruitment after consultation with and approval from the CA.

7.2 Discontinuation of IMP

- Subjects who receive or require any of the following interventions during treatment period will be discontinued from study treatment:
  - Renal replacement therapy (RRT) i.e. dialysis
  - Intra-operative and/or post-operative intra-aortic balloon pump (IAPB)
  - Percutaneous or surgical mechanical circulatory support (MCS) or extra-corporeal membrane or oxygenator (ECMO)
- Study treatment will also be stopped if any of the following liver chemistry stopping criteria, defined in the FDA Guidance on Drug-Induced Liver Injury[31] is met:
  - ALT ≥ 3 x Upper Limit of Normal (ULN) combined with total bilirubin ≥ 2 x ULN (>35% direct bilirubin).
    NOTE: serum bilirubin fractionation should be performed if testing is available. If fractionation is unavailable, urinary bilirubin is to be measured via dipstick (a measurement of direct bilirubin, which would suggest liver injury).
  - ALT ≥ 3 x ULN combined with International Normalized Ratio (INR) > 1.5
  - ALT ≥ 5 x ULN.
  - ALT ≥ 3 x 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).
- In rare instances, it may be necessary for a subject to prematurely discontinue (definitive discontinuation) study treatment. If study treatment is definitively discontinued, the subject will remain in the study to be evaluated until visit 7 (if possible) or complete visit 7 as an early termination visit as a minimum.
- Subjects who develop a grade 3 or higher Adverse Event as per CTCAE v5.0 Nov 2017 [4], which is an Injection Site Reaction or Infusion Related Reaction and which is considered to be an immune mediated reaction, will discontinue IMP.
- Subjects who develop stage 3 (see section 8.6) AKI, according to KDIGO definition [5]

7.3 Subject Discontinuation/Withdrawal from the Study

- 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, behavioural, compliance, or administrative reasons. This is expected to be uncommon.
- 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 assessments performed according to visit 7 (e.g. the end of study visit).
  See the Schedule of Assessments (Section 1.2) for data to be collected and transcribed to the eCRF. Any ongoing SAE at the time of withdrawal will be followed as described in Section 8.4.
- 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 eCRF.
- Discontinuation of the study as its whole is handled as part of Appendix 1.

7.3.1 Replacement of subjects

- Up to two (2) additional subjects may be randomised in case of early discontinuations for other reasons than formal stopping criteria before visit 4. This is to ensure 12 subjects completing visit 4 in the study. Subjects replacing a discontinued subject should be randomised to the same treatment arm to maintain a balanced randomisation.

8 STUDY ASSESSMENTS AND PROCEDURES

- The study assessments and procedures are described below and the timing of the same are summarized in the Schedule of Assessments (Section 1.2).
- Adherence to the study design requirements, including those specified in the Schedule of Assessments, is essential and required for study conduct and protocol waivers or exemptions are not allowed.
- Immediate safety concerns should be discussed with the sponsor immediately upon occurrence or awareness to determine if the subject should continue or discontinue the study.
- All subjects screened will be assigned a screening number and the investigator will maintain a screening log to record details of all subjects screened and to confirm eligibility or record reasons for screening failure, as applicable.
- All screening evaluations must be completed and reviewed to confirm that potential subjects meet all eligibility criteria.
- Subjects found eligible will be randomised to receive one of the two treatment arms according to a computer-generated randomization list provided by KLIFO A/S.
- The planned maximum amount of blood collected from each subject over the duration of the study, will not exceed approximately 400 mL. Repeat or unscheduled samples may be taken in addition for safety reasons or for technical issues with the samples.

8.1 Recording of Data

The Principal Investigator will provide the Sponsor with all data produced during the study from the scheduled assessments and ensure the accuracy, completeness, legibility and timeliness (within 7 days) of the data reported to the Sponsor in the eCRF and in all required reports.

8.2 Demographics and other baseline characteristics

8.2.1 Informed Consent

Signed Informed Consent must be obtained before any screening procedures are initiated. For further instruction on the procedure, see Section 10.1.3.

8.2.2 Demography

The following demography data will be collected and recorded: gender, age (years), ethnicity (Hispanic or Latino, non-Hispanic or Latino) and race (American Indian or Alaska Native, Asian, Black or African American, Native Hawaiian or Other Pacific Islander, White).

8.2.3 Height and weight

Height (cm) and weight (kg with one decimal) will also be measured and recorded.
The weight recorded at the screening visit will be used for calculation of IMP dose.

8.2.4 Medical/surgical history

Medical/surgical history and disease characteristics, a listing of relevant past and current diseases, and the details of active diseases will be obtained from medical records.

8.2.5 Prior and concomitant medication

Prior prescription and non-prescription medications (e.g. over-the-counter drugs and herbal supplements) taken within 28 days prior to screening will be obtained by the Principal Investigator by interview and/or medical history.

Medications are classified as prior if the stop date is before or on the day for obtaining Informed Consent.

Concomitant medication refers to all drugs and therapies used from the time the ICF was signed through the end of study participation and is described in Section 6.5.

Medications (incl. anaesthesia) given before and during surgery will be recorded and transferred to the eCRF.

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

8.2.6 Pregnancy test

All female subjects of child-bearing potential will have a serum pregnancy test performed at screening as pregnant subjects will be excluded to participate in the study. In addition, serum pregnancy tests must be performed at the end of study visit (Visit 7).
For specific tests, see Appendix 2.

8.3 Safety Assessments

Timing and frequency of all safety assessments are outlined in the Schedule of Assessments (Section 1.2).

Any abnormalities in the safety assessment will be specified and documented as clinically significant or not clinically significant. Abnormal post-dose findings assessed by the Investigator as clinically significant will be reported as AEs.
Any abnormal laboratory test results (hematology, clinical chemistry or urinalysis) or other safety assessments (e.g. ECG, continuous ECG monitoring, vital signs measurements), including those that worsen from baseline, considered clinically significant in the medical and scientific judgment of the investigator (i.e. not related to progression of underlying disease or medical procedure, i.e. cardiac surgery) is an Adverse Event (Section 10.3.1).

8.3.1 Physical Examinations

A complete physical examination will include, at a minimum, assessments of the following systems:

- Cardiovascular
- Neurological
- Head (including ears, nose, throat [ENT], and eyes)
- Lymphatic system
- Endocrine system
- Peripheral vascular system
- Lungs and respiratory tract
- Gastrointestinal, liver, spleen
- Urogenital system
- Muscular and skeletal system

- Skin and connective tissue

8.3.2 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 eCRF on Visit 2.
Data points to collect are time on CPB, duration of surgery, blood loss volume, administration of fluids (red blood cells, crystalloids, colloids and other) and time of admission to the ICU, see Section 1.2.

8.3.3 Vital Signs

- Vital signs will include body temperature (˚C (one decimal)), systolic and diastolic blood pressure (BP) (mmHg), heart rate (bpm) and respiratory rate (breaths per min).
- Vital signs will be measured in a supine position for the subject preceded by at least 5 minutes rest in a quiet setting without distractions (e.g. television, cell phones).
- BP, heart rate and respiratory rate measurements will be assessed by three consecutive readings recorded at intervals of at least 1 minute. The average of the three readings will be recorded in the CRF.

8.3.4 Electrocardiograms

- Single 12-lead ECG will be obtained in supine position after 10 minutes of rest using an ECG machine that automatically calculates the heart rate and measures PR, QRS, QT, and QTc intervals.
- 12-lead ECGs will be reviewed on-site by the Investigator.

8.3.5 ECG monitoring

- Continuous ECG monitoring will be used for cardiac surveillance from start of first infusion until 6 hours after start of last IMP administration and will be monitored on-site by the Investigator.
- Data will be monitored but not recorded in the eCRF.

8.3.6 Clinical Safety Laboratory Assessments

Clinical chemistry, cardiac biomarkers and hematology

- Blood samples for analysis of clinical chemistry (including Creatinine, cystatin C and liver function tests), cardiac biomarkers and hematology parameters will be collected through venipuncture or an indwelling venous catheter.
- See Appendix 2 for the list of clinical laboratory tests to be performed and the Schedule of Assessments (Section 1.2) for the timing and frequency.
- Actual sampling times will be recorded.
- The analysis of the samples will be performed by the certified clinical chemistry laboratory at the University Hospital in Münster according to routine analytical methods.
- The investigator must review the laboratory report, document the result of the review (i.e. clinical assessment for values out of range) on the laboratory report, and record any clinically relevant changes occurring during the study in the AE section

of the eCRF. 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 with values considered clinically significantly abnormal during participation in 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 (e.g. SAE or AE), then the results must be recorded in the eCRF.

Urinalysis

- Urinalysis by means of a regular dipstick test will be performed at the investigational site.
- See Appendix 2 for the list of clinical laboratory tests to be performed and the Schedule of Assessments (Section 1.2) for the timing and frequency of sampling.
- Actual sampling times and results for each parameter will be recorded in the eCRF.

8.3.7 Urinary output

Collection of urine according to site standard procedures will start before surgery via a urine catheter and continue until 48 hours after start of first IMP administration. The urinary output (mL per hour) will be recorded, as stated in the Schedule of Assessments (Section 1.2). Urine will be collected from spontaneous voids in case the catheter is removed before the collection ends. Total volume (mL) of urinary output during each collection period will be measured, recorded and transferred to the eCRF.
Urine volume collected before IMP administrations 3-5 will be used together with a urine Creatinine value to calculate the CrCl (i.e. renal function), as specified in Schedule of Assessment (Section 1.2). The CrCl will decide if the dose for the coming IMP administration should be reduced.

8.4 Adverse Events and Serious Adverse Events

See further guidance in Section 10.3: Adverse Events: Definitions and Procedures for Recording, Evaluating, Follow-up, and Reporting

AEs will be reported by the subject (or, when appropriate, by a caregiver or surrogate).

The investigator and any qualified designees are responsible for detecting, documenting, and recording events that meet the definition of an AE or SAE and remain responsible for following up AEs that are serious, considered related to the study intervention or study procedures, or that caused the subject to discontinue the study (Section 7.2)

8.4.1 Time Period and Frequency for Collecting AE and SAE Information

All AEs and SAEs will be collected from the signing of the informed consent form (ICF) until end of study-visit at the time points specified in the Schedule of Assessments (Section 1.2).

All SAEs will be recorded and reported to KLIFO Pharmacovigilance (PV) and sponsor immediately and under no circumstance should this exceed 24 hours, as indicated in Appendix 3. The investigator will submit any updated SAE data to KLIFO within 24 hours of it being available.

Investigators are not obligated to actively seek AE or SAE after conclusion of the study participation. 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 study intervention or study participation, the investigator must promptly notify the sponsor.

8.4.2 Method of Detecting AEs and SAEs

The method of recording, evaluating, and assessing causality of AEs and SAEs and the procedures for completing and transmitting SAE reports are provided in Appendix 3.

Care will be taken not to introduce bias when detecting AEs and/or SAEs. Open-ended and non-leading verbal questioning of the subject is the preferred method to inquire about AE occurrences.

8.4.3 AEs and SAEs excluded from recording and reporting

A pre-existing condition (i.e., a disorder present before the AE reporting period started/ signing the Informed Consent Form (ICF) recorded on the medical history/physical examination form) should not be recorded as an AE unless the condition worsens or episodes increase in frequency during the AE reporting period.

Signs and symptoms which, according to the investigator are expected and well established and known consequences of the indication or the surgical procedure, both in intensity and frequency, should not be recorded as AEs or SAEs except for events with a fatal outcome. Any unexpected change in the intensity or frequency must be recorded and reported as an AE or SAE as applicable.

8.4.4 Follow-up of AEs and SAEs

After the initial AE/SAE report, the investigator is required to proactively follow each subject at subsequent visits/contacts. All SAEs including AEs of Special Interest (as defined in Section 8.4.7), must be followed on a regular basis, according to the investigator's clinical judgment, until the event has resolved or until the investigator assesses it as chronic or stable. This implies follow up may continue after EOT and that additional investigations may be requested by Sponsor. Further information on follow-up procedures is provided in Appendix 3.

8.4.5 Regulatory Reporting Requirements for SAEs

- Prompt notification by the investigator to the KLIFO PV of a SAE is essential so that legal obligations and ethical responsibilities towards the safety of subjects and the safety of a study intervention under clinical investigation are met.
- The sponsor has a legal responsibility to notify both the local regulatory authority and other regulatory agencies about the safety of a study intervention under clinical investigation. The sponsor will comply with country-specific regulatory

requirements relating to safety reporting to the regulatory authority, Independent Ethics Committees (IEC), and investigators.

- For all studies investigator safety reports must be prepared for suspected unexpected serious adverse reactions (SUSAR) according to local regulatory requirements and sponsor policy and forwarded to the regulatory authority, Independent Ethics Committees (IEC), and investigators as necessary.
- An investigator who receives an investigator safety report describing a SAE or other specific safety information (e.g. summary or listing of SAEs) from the sponsor will review and then file it along with the Investigator's Brochure.

8.4.6 Pregnancy

- Details of all pregnancies in female subjects and, if indicated, female partners of male subjects will be collected after the start of treatment period and until end of study visit.
- If a pregnancy is reported, the investigator should inform the sponsor within 24 hours of learning of the pregnancy and should follow the procedures outlined in Appendix 4.
- Abnormal pregnancy outcomes (e.g. spontaneous abortion, fetal death, stillbirth, congenital anomalies, ectopic pregnancy) are considered SAEs.

8.4.7 Adverse Events of Special Interest

The following events are to be regarded Adverse Events of Special Interest (AESI), should they occur:

- Injection Site Reaction (ISR) or Infusion Related Reaction (IRR) adverse events.

All AESIs will be recorded and reported

- as diagnosis with description of all localized and generalized symptoms
- according to SAE timelines, regardless of whether the event is non-serious or serious (see section 8.4.1).

The CTCAE ISR and IRR definitions and grading classifications are included in Section 10.3.3.

8.5 Treatment of Overdose

An overdose is a dose higher than the dose specified in the protocol.

Overdosing is not likely to occur in this study as all IMP will be administered at the Investigational site under medical surveillance.

Sponsor does not recommend specific treatment for accidental overdoses, supportive measures as per standard of investigational site should take place (see Section 2.3). No known antidote is available.

In the event of an overdose, the investigator should:

1. Contact the sponsor immediately.
2. Closely monitor the subject for any AE/SAE and laboratory abnormalities until the IMP can no longer be detected systemically.

3. Document the quantity of the excess dose as well as the duration of the overdose in the eCRF.

Decisions regarding dose interruptions or modifications will be made by the investigator in consultation with the Sponsor based on the clinical evaluation of the subject.

8.6 Efficacy Assessments

Parameters from efficacy (and safety) assessments will be based on post-baseline changes in following parameters:

Efficacy parameters	**Definition**
Biomarkers of tubular stress/damage, oxidative stress, inflammatory cytokines, cardiac stress/function (see Section 8.8)	Post-baseline changes
Hemodynamic assessments (cardiac output (CO) (using echocardiography), mixed venous saturation (SvO2) and central venous pressure (CVP) (measured in central venous line).	Post-baseline changes
SCr and Cystatin C	• Post-baseline changes in actual values • Percent increase and maximum percent increase in SCr and cystatin C (and corresponding estimated GFR values based on CKD-EPI and modified CKD-EPI equations, respectively) from baseline • Post-baseline AUC of SCr and Cystatin C levels (and corresponding eGFR values) from baseline to 48 hours, 72 hours and 7 days post-surgery
Major Adverse Kidney Event	Present at visit 7 (MAKE30), assessed as developed if any of the following have occurred: • Death • Dialysis • Relative reduction of eGFR ≥25% Assessment is based on either CKD-EPI or modified CKD-EPI equations.
Severity of AKI (using biochemistry result of SCr and urinary output measurements)	Grade based on KDIGO definition [5]: • Stage 1: SCr 1.5 to 1.9 times baseline within 7 days, OR ≥0.3 mg/dL (≥ 26.5 µmol/L) within 48 hours OR urine output <0.5 mL/min/h for 6 to<12 hours • Stage 2: SCr 2.0-2.9 times baseline within 7 days OR urine output <0.5 mL/min/h for ≥ 12 hours • Stage 3: SCr 3.0 times baseline within 7 days, OR increase in SCr 5.0 mg/dL (≥353.6 µmol/L), OR initiation of renal replacement therapy OR urine output <0.3 mL/min/h for ≥24 hours OR anuria for ≥12 hours
Duration of AKI (using biochemistry result of SCr and urinary output measurements)	Duration based on KDIGO definition: The number of days from start of AKI (KDIGO definition) where either SCr increase ≥ 0.3 mg/dL above pre-AKI reference point (first comparison is versus pre-surgery

	baseline, or a reference value after baseline if AKI occurs after 48 hours post-surgery) OR if SCr increase is ≥ 1.5 times baseline or dialysis in the first 7 days, OR up to discharge if prior to 7 days. Persistent AKI defined as AKI (KDIGO definition) for >48 hours
Length of Index ICU stay (days)	Duration of stay (number of days) in the ICU immediately following surgery or recovery room post-surgery until discharge
Length of Index Hospital stay (days)	Duration of stay in the hospital from admission to hospital discharge for the index surgery
Nature of patient discharge facility	I.e. home, skilled nursing facility, rehabilitation center, other

The assessments will be performed at the Investigational Site.

8.7 Pharmacokinetics 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 venous line.

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

Samples will be taken at the pre-specified time points in Table 4 and in Schedule of Assessments (Section 1.2):

Table 4 Plasma PK: Sampling time point's incl. allowed sampling windows

Plasma PK sampling time window	**Predose** ≤30min	**15 min** ±5min	**30 min** ±5min	**1 h** ±5min	**2 h** ±15min	**3 h** ±15min	**4 h** ±15min	**5 h** ±15min
Start of Infusion 1 (t = 0 h)	X	X	X	X	X	X	X	X
Start of Infusion 2 (t = 6 h)	X		X	X	X			
Start of Infusion 3 (t = 12 h)	X		X	X*	X*			
Start of Infusion 4 (t = 24 h)	X		X	X	X	X	X	X
Start of Infusion 5 (t = 48 h)	X		X	X	X			

* Optional PK sampling times

Urine samples for the determination of urine concentrations of RMC-035 after administration of the IMP, will be collected as part of sampling of exploratory biomarkers at predefined time points as the urine biomarkers (see Table 5).

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

Plasma samples for determination of concentrations of RMC-035 will be analysed by Mercodia AB by means of a validated enzyme linked immunosorbent assay (ELISA). Urine samples for determination of RMC-035 concentration will be analysed by Mercodia AB or by the Sponsor.
The PK parameters in plasma and urine to be evaluated are listed in Section 9.4.5.

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

8.8 Pharmacodynamics and Biomarkers

Collection of blood and urine samples for biomarker research is also part of this study.

- Blood samples will be collected through an arterial line or an indwelling 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.

Samples for the following biomarker categories will be collected from all subjects in this study as specified in the Schedule of Assessments
(see Section 1.2) and Table 5:

- Tubular injury and stress markers e.g.
  - KIM-1, TIMP2, IGFBP7, IL-18, L-FABP, NGAL (urine)
  - KIM-1, NGAL and A1M (plasma)

- Oxidative stress markers e.g.
  - 8OH-dG (serum and urine)

- Inflammatory cytokines e.g.
  - IL-8 and IL-6 (plasma)

Additional biomarkers within these categories may be measured from already collected samples.

Table 5 Biomarkers: sample, assay and time points' incl. allowed sampling windows

Biomarker	**Sample**	**Assay**	**Predose** ≤60min	**4 h** ±15min	**12 h** ±15min	**24 h** ±30min	**48 h** ±30min	**72 h** ±60min
A1M	Serum	ELISA	x	x	x	x	x	x
KIM-1	Serum	ELISA, multiplex	x	x	x	x	x	x
NGAL	Plasma	ELISA, multiplex	x	x	x	x	x	x
8OH-dG	Serum	ELISA	x	x	x	x	x	x
Inflammatory cytokines (eg. IL-8, IL-6)	Plasma	ELISA, multiplex	x	x	x	x	x	x
KIM-1	Urine	ELISA, multiplex	x	x	x	x	x	x
NGAL	Urine	ELISA, multiplex	x	x	x	x	x	x
8OH-dG	Urine	ELISA	x	x	x	x	x	x
TIMP2 / IGFBP7	Urine	Nephro-check	x	x	x	x	x	x
IL-18	Urine	ELISA, multiplex	x	x	x	x	x	x
L-FAPB	Urine	ELISA, multiplex	x	x	x	x	x	x
RMC-035	Urine	ELISA	x	x	x	x	x	x

Samples will be analysed, stored and destroyed at the investigational site according to the normal procedures at the site.

8.9 Immunogenicity Assessments

Blood samples for the determination of anti-drug antibodies (ADA) after administration of IMP will be collected through an indwelling venous catheter or venipuncture as detailed in the Schedule of Assessments (see Section 1.2).

Instruction on the sample collection and handling will be detailed in the laboratory manual or equivalent.

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 taking into account 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 will be analysed by Wieslab AB.

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 may be further characterized beyond the scope of the study.

8.10 Medical Resource Utilization and Health Economics

This section is not relevant for this study as medical resource utilization and health economics will not be studied.

9 STATISTICAL CONSIDERATIONS

9.1 Statistical Hypotheses

No statistical hypotheses are planned to be tested in this study.

9.2 Sample Size Determination

No formal sample size calculation has been performed for this study. The number of subjects is based on precedent set by other clinical studies of similar nature.
The proposed sample size of 12 evaluable subjects (8 subjects on active treatment and 4 on placebo) is considered sufficient to achieve the clinical study objectives.

9.3 Population for Analyses

The following populations are defined:

Table 6 Defined Populations

Population	Description
Randomised	All subjects who have been randomised at Day -1
Full Analysis Set (FAS)	The Full Analysis Set will consist of all randomised subjects who have received at least 1 dose of IMP. FAS will be used for efficacy and safety endpoints.
PK Analysis Set (PK)	PK Analysis Set will be used for the PK evaluation and will consist of all randomised subjects with no relevant protocol violations affecting the evaluation of the PK parameters.

9.4 Statistical Analyses

The statistical analysis plan (SAP) will be signed and approved prior to database lock (DBL) and it will include a more technical and detailed description of the statistical analyses described in this section. This section is a summary of the planned statistical analyses of the most important endpoints.

9.4.1 General Considerations

All statistical analyses will be descriptive. Details of the data summaries will be presented in the SAP.

Statistical analyses will be conducted after the end of the study (last patient end of study visit) once the database has been locked and data is unblinded. The results will be included in the clinical study report (CSR).

Changes from the statistical analysis planned in this protocol will be described and justified in a protocol amendment, in the SAP, and/or in the CSR, as applicable, dependent on the type of deviation.

All statistical analysis will be performed using SAS®.

9.4.2 Demographic and Baseline Characteristics

- Demography data, medical/surgical history and all other relevant background data will be summarized in terms of descriptive statistics.

9.4.3 Treatment compliance

- The number of subjects treated in each treatment group will be presented by treatment in listings.

9.4.4 Primary endpoint(s)

Safety analyses will be made on FAS.

- Adverse Events; frequency (number and percentage) of subjects with TEAEs and AEs per treatment group will be fully listed by Investigator terms and MedDRA Preferred Term (PT). They will furthermore be summarized by System Organ Class (SOC) and PT.

9.4.5 Secondary endpoint(s)

All safety analyses will be made on FAS.

- Physical examination; abnormal findings will be specified and presented by subject and summarized by treatment group
- Vital signs; systolic/diastolic blood pressure, respiratory rate, heart rate and body temperature will be summarized by descriptive statistics by visit and as changes from baseline to each visit split by treatment group
- 12-lead ECG; will be listed for each subject and summarized as the vital signs parameters. ECGs will be categorized as “normal”, “abnormal, not clinically significant” or “abnormal, clinically significant” (as judged by the Investigator) and summarized by treatment group using frequency tables and listings
- Safety laboratory analyses data (hematology, clinical chemistry incl. liver function test, urinalysis); will be presented with descriptive statistics by individual time courses and as change from baseline for each parameter and summarized by treatment group using listings.

Descriptive statistics will be presented for plasma concentrations by scheduled sample time.

Calculation of pharmacokinetic variables:

The PK analysis will be based on the PK set.
The PK parameters will be calculated by non-compartmental analysis (NCA) using software Phoenix WinNonlin®.

The following PK parameters in plasma will be assessed:

- C_{max} (maximum observed concentration)
- C_{trough} (lowest concentration reached before next dose is administered)
- AUCτ (area under the curve from 0 to t, where t is the last measurable sample after **fourth** dose)
- Peak to trough ratio (PTR) (after **fourth** dose)
- $R_{ac\ (AUC)}$ (Accumulation ratio calculated from AUCτ,ss and AUCτ after single dosing)
- $t_{½}$ (terminal half-life after **fourth** dose)
- PK parameters will also be calculated or extrapolated after all other dose administrations

Listings for the PK parameters will be presented separately by treatment group, age group (18-69 years of age and ≥70 years of age) and renal function at baseline. The parameters will also be summarized with number of measurements, arithmetic mean, SD, CV, median,

minimum, maximum, geometric mean, geometric CV%. In addition, the individual and mean concentration-time profiles will be illustrated graphically. Dose proportionality will be evaluated for C_{max} and AUCτ.

Concentration values below the lower limit of quantitation (LLOQ) that precede the first quantifiable concentration value will be set to zero for linear plots and for all calculations and summary statistics but will be excluded from semi-logarithmic plots. All other LLOQ values will be treated as missing for all analyses. Possible outlying concentration values excluded from the PK analysis will be reported and justified in the study report.

Analysis of presence and titers of ADA will be performed on FAS.

Based on the relatively small sample size no formal hypothesis testing will be conducted.

9.4.6 Exploratory endpoint(s)

Efficacy/pharmacodynamics/biomarkers as well as urine concentration of RMC-035 (normalized for urine creatinine) assessments are part of exploratory endpoints only and will be performed on FAS and summarized using frequency tables and descriptive statistics for absolute and/or relative change from baseline.

9.5 Interim Analyses

No interim analyses will be performed.

9.6 Data Monitoring Committee (DMC)

No data monitoring committee will be established and used in this clinical study.

10 SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

10.1 Appendix 1: Regulatory, Ethical, and Study Oversight Considerations

10.1.1 Regulatory and Ethical Considerations

- This study will be conducted in accordance with the protocol and with the following:
  - Consensus ethical principles derived from international guidelines including the Declaration of Helsinki and Council for International Organizations of Medical Sciences (CIOMS) International Ethical Guidelines
  - Applicable ICH Good Clinical Practice (GCP) Guidelines
  - Applicable laws and regulations
- The protocol, ICF, Investigator Brochure, and other relevant documents (e.g. advertisements) must be submitted to an IEC and as applicable to the Competent Authority (CA) and reviewed and approved by the IEC and CA before the study is initiated.
- Any amendments to the protocol will require IEC and CA approval before implementation of changes made to the study design, except for changes necessary to eliminate an immediate hazard to study subjects.
- KLIFO will be responsible for the following:
  - Notifying the IEC and CA of SUSARs or other significant safety findings as required by local law.
  - Providing oversight of the conduct of the study at the site and adherence to requirements of ICH guidelines, the IEC, European Directive 2001/20/EC, European regulation 536/2014 for clinical studies (if applicable), and all other applicable local regulations
- The Sponsor has delegated the responsibility for submission of study documents to IEC and CA to KLIFO.

10.1.2 Financial Disclosure

Principal Investigator and all physicians 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.

10.1.3 Informed Consent Process

- The investigator will explain the nature of the study to the subject and answer all questions regarding the study.
- Subjects must be informed that their participation is voluntary and they may withdraw from the study at any time.
- Subjects will be required to sign and personally date a statement of informed consent that meets the requirements of ICH-GCP requirements, where applicable, and the IEC or investigational site after the subject has had sufficient amount of time to consider participation.

- 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.
- Subjects must be re-consented to the most current version of the ICF(s) during their participation in the study.
- A copy of the ICF(s) must be provided to the subject.

10.1.4 Data Protection

- Subjects will be assigned a unique identifier by the sponsor. Any subject records or datasets that are transferred to the sponsor will contain the identifier only, i.e. data will be transferred in a pseudonymized manner. Subject names or any information which would make the subject identifiable will not be disclosed. A Subject Identification List or equivalent will be kept at the investigational site and no one outside of the investigational site will be able to identify the participating subjects.
- 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 who will be required to give consent for their data to be used as described in the informed consent.
- The subject must be informed that his/her medical records may be examined by Clinical Quality Assurance (QA) auditors or other authorized personnel appointed by the sponsor and by inspectors from regulatory authorities.
- All data will be handled in accordance with the EU's current Data Protection Law Enforcement Directive, The General Data Protection Regulation (GDPR) and the local data protection regulations.
- For this study, the Sponsor and Investigational Site are the data controller of all data processed during the study and KLIFO and other sub-contractors used are data processors.

10.1.5 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.

10.1.6 Dissemination of Clinical Study Data

Basic information about this study will be registered in the global data registry www.clinicaltrials.gov before the first patient enters into the trial.

A clinical study report describing the conduct of the study, the statistical analysis performed and the results obtained, will be prepared by the sponsor or delegate. The report will be reviewed and approved by, as a minimum, the investigators, the statistician and the sponsor. The study results will be reported in the European Clinical Trials Database (EudraCT) database per applicable regulations within 12 months after completion of the trial.

A summarising report will be submitted to the applicable CA and IEC within 12 months after completion of the trial.

10.1.7 Data Quality Assurance

- All subject data relating to the study will be recorded in an electronic eCRF unless transmitted to the sponsor or designee electronically (e.g. laboratory data). The investigator is responsible for verifying that data entries are accurate and correct by physically or electronically signing the eCRF.
- The investigator must maintain accurate documentation (source data) that supports the information entered in the eCRF.
- Monitoring details describing strategy (e.g. risk-based initiatives in operations and quality such as Risk Management and Mitigation Strategies and Analytical Risk-Based Monitoring), methods, responsibilities and requirements, including handling of noncompliance issues and monitoring techniques (central, remote, or on-site monitoring) are provided in the Monitoring Plan.
- Study monitors will perform ongoing source data verification (SDV) to confirm that data entered into the eCRF 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.
- The investigator must permit study-related monitoring 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.
- The sponsor assumes accountability for actions delegated to other individuals (e.g. Contract Research Organizations (CRO)).
- The sponsor and CRO have the right to perform an audit of the investigational site and the sponsor may also audit the CRO. Such audits will be conducted according to a specific audit plan.
- The regulatory (competent) authorities, both national and foreign, may inspect the investigational site at any time. The investigator is responsible for notifying the sponsor of such an inspection immediately after gaining knowledge of it.
- During the audit or inspection, the investigator will permit the auditor, and regulatory inspector(s) direct access to all relevant medical records and other source data, study-related files and eCRFs.
- Records and essential documents, including signed ICFs, pertaining to the conduct of this study must be retained by the investigator for a minimum of 15 years after completion or discontinuation of the trial, if no further instructions are given by the sponsor. No records may be destroyed during the retention period without the written approval of the sponsor. No records may be transferred to another location or party without written notification to the sponsor.

10.1.8 Source Documents

- Source documents provide evidence for the existence of the subject and substantiate the integrity of the data collected. Source documents are filed at the investigator's site.
- Data entered in the eCRF 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. Also, current medical records must be available.
- After data entry, the AEs will be coded according to MedDRA. Prior and Concomitant Medication will be coded according to WHO Drug Reference List and Anatomical Therapeutic Chemical Classification System (ATC). Medical History will be coded according to MedDRA.
- Definition of what constitutes source data and where source data is located can be found in a Source Data Agreement Form or equivalent.

10.1.9 Study and Site Start and Closure

The study start date is the date on which the clinical study will be open for recruitment of subjects at the investigational site and the study will be closed after the last subject has commenced last visit.

Planned trial period:

First Subject First Visit: Q4 2020

Last Subject Last Visit: Q3 2021

The sponsor or designee 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. An investigational 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 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 an investigational 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 IEC or local health authorities, the sponsor's procedures, or GCP guidelines
- Inadequate recruitment of subjects by the investigator
- Discontinuation of further development of the IMP
- If the study is prematurely terminated or suspended, the sponsor shall promptly inform the Investigators, the IECs, the regulatory authorities, and any contract research organization(s) used in the study of the reason for termination or suspension, as specified by the applicable regulatory requirements. The Investigator shall promptly inform the subject and should assure appropriate subject therapy and/or follow-up.Publication Policy
- The results of this study may be published or presented at scientific meetings. If this is foreseen, the investigator agrees to submit all manuscripts or abstracts to the

sponsor before submission. This allows the sponsor to protect proprietary information and to provide comments.

- The sponsor will comply with the requirements for publication of study results. In accordance with standard editorial and ethical practice, the sponsor will generally support publication of studies with many investigational sites only in their entirety and not as individual site data. In this case, a coordinating investigator will be designated by mutual agreement.
- Authorship will be determined by mutual agreement and in line with International Committee of Medical Journal Editors authorship requirements.
- External CRO or laboratory involved in the conduct of the study has no publication rights regarding this study.

10.2 Appendix 2: Clinical Laboratory Tests

- The tests detailed below will be performed by the local laboratory at the University Hospital in Münster.
- Additional tests may be performed at any time during the study as determined necessary by the investigator.
- Pregnancy Testing at screening and at Visit 7 only apply for WOCBP.

Table 7 Protocol Required Safety Laboratory Assessments

Laboratory Assessments	Parameters		
Hematology	Erythocyte Volume Fraction (EVF)	Mean corpuscular volume (MCV)	Monocytes
	Hemoglobin (Hb)	Mean corpuscular hemoglobin (MCH)	Lymphocytes
	Thrombocytes	Mean corpuscular hemoglobin concentration (MCHC)	Eosinophils
	Erythocytes	Leucocytes	Basophils
		Neutrophils	
Clinical Chemistry (excl. liver function labs)	Albumin	Cystatine C	Sodium
	Calcium	Estimated glomerular filtration rate (based on creatinine and cystatin C)	Urea nitrogen
	Chloride	Magnesium	Uric acid
	Serum Creatinine	Phosphate	
	C-reactive protein (CRP)	Potassium	
Liver function lab	Alanine Aminotransferase (ALT)	Aspartate Aminotransferase (AST)	Gamma glutamyltransferase (GGT)
	Alkaline phosphatase (ALP)	Bilirubin (total and conjugated)	
Cardiac biomarkers	NT-proBNP	Troponin-I	
Urinalysis	Specific gravity	pH	Leucocytes
	Erythrocytes	Protein	Glucose
	Nitrite	Urobilinogen	Bilirubin
	Ketones	Microscopic examination (if blood or protein is abnormal)	
Urine test	Creatinine		
Other Screening Tests	• Highly sensitive serum human chorionic gonadotropin (hCG) serum pregnancy test (as needed for WOCBP) • Hemoglobin A1c (HbA1c) in blood		

10.3 Appendix 3: Adverse Events: Definitions and Procedures for Recording, Evaluating, Follow-up, and Reporting

10.3.1 Definition of AE

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

Events <u>Meeting</u> 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 (i.e., not related to progression of underlying disease). • 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 <u>NOT</u> 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 (e.g. 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.

10.3.2 Definition of SAE

If an event is not an AE per definition above, then it cannot be an SAE even if serious conditions are met (e.g. hospitalization for signs/symptoms of the disease under study, death due to progression of disease).

A SAE is defined as any untoward medical occurrence that, at any dose:
a. Results in death
b. 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.
c. 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.
d. 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 (e.g. sprained ankle) which may interfere with or prevent everyday life functions but do not constitute a substantial disruption.
e. Is a congenital anomaly/birth defect
f. Other situations: • 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.

10.3.3 Recording and Follow-Up of AE and/or SAE

AE and SAE Recording

- When an AE/SAE occurs, it is the responsibility of the investigator to review all documentation (e.g. hospital progress notes, laboratory reports, and diagnostics reports) related to the event.
- The investigator will then record all relevant AE/SAE information in the eCRF.
- It is **not** acceptable for the investigator to send photocopies of the subject's medical records to KLIFO PV in lieu of completion of the AE/SAE eCRF page.
- There may be instances when copies of medical records for certain cases are requested by KLIFO PV. In this case, all subject identifiers, with the exception of the subject number, will be redacted on the copies of the medical records before submission to KLIFO PV.
- The investigator will attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. Whenever possible, the diagnosis (not the individual signs/symptoms) will be documented as the AE/SAE.

Assessment of severity (CTCAE grade)

The investigator will make an assessment of severity for each AE and SAE reported during the study and assign it to grades 1 through 5 according to the CTCAE classification version 5.0. [4]

Grade refers to the severity of the AE. The CTCAE displays grades 1 through 5 with unique clinical descriptions of severity for each AE based on this general guideline:

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

Grade 2: Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL*.

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.

A Semi-colon indicates 'or' within the description of the grade.

Activities of Daily Living (ADL)

*Instrumental ADL refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.

**Self care ADL refer to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.

Injection Site Reaction (ISR) or 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 deadline described in section 8.4.1. The CTCAE grading for ISR and IRR are provided in the table below. For unique clinical descriptions of severity for each AE, refer to the CTCAE classification guideline.

CTCAE Term	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5	Definition
Injection site reaction (ISR) MedDRA SOC: General disorders and administration site conditions	Tenderness with or without associated symptoms (e.g., 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.
Infusion related reaction (IRR) 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 (e.g., antihistamines, NSAIDS, narcotics, IV fluids); prophylactic medications indicated for <=24 hrs	Prolonged (e.g., 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.

An event is defined as 'serious' when it meets at least 1 of the predefined outcomes as described in the definition of an SAE, NOT when it is rated as severe.

Assessment of Causality

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

- Alternative etiology should be provided for all AEs assessed as possible 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 Investigator's Brochure (IB) version 6 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 to KLIFO PV. However, it is very important that the investigator always make an assessment of causality for every event before the initial transmission of the SAE data to KLIFO PV.
- 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.

Assessment of Outcome

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 (e.g. became blind, deaf, paralysed). 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.

Follow-up of AEs and SAEs

- The investigator is obligated to perform or arrange for the conduct of supplemental measurements and/or evaluations as medically indicated or as requested by Sponsor to elucidate the nature and/or causality of the AE or SAE as fully as possible. This may include additional laboratory tests or investigations, histopathological examinations, or consultation with other health care professionals.

- If a subject dies during participation in the study or during a recognized follow-up period, the investigator will provide KLIFO PV with a copy of any post-mortem findings including histopathology.
- New or updated information will be recorded in the originally completed eCRF.
- The investigator will submit any updated SAE data to KLIFO PV within 24 hours of receipt of the information.

10.3.4 Reporting of SAEs

SAE Reporting to KLIFO PV via the eCRF

- The primary mechanism for reporting an SAE to KLIFO PV will be the eCRF.
- If the electronic system is unavailable, then the site will use the paper SAE data collection tool (see next section) in order to report the event within 24 hours.
- The site will enter the SAE data into the eCRF as soon as it becomes available.
- After the study is completed at a given site, the eCRF will be taken off-line to prevent the entry of new data or changes to existing data.
- If a site receives a report of a new SAE from a study subject or receives updated data on a previously reported SAE after the eCRF has been taken off-line, then the site can report this information on a paper SAE form (see next section).

SAE Reporting to KLIFO PV via Paper SAE form (in case of eCRF unavailability)

- E-mail or fax transmission of the SAE via a paper form is the preferred method to transmit this information to KLIFO PV.
- Initial notification via e-mail or fax does not replace the need for the investigator to enter the SAE data into the eCRF as soon as it becomes available.
- Contacts for e-mail or fax SAE reporting:
  - Email: pharmacovigilance@klifo.com
  - Fax: +45 39 209 045

10.4 Appendix 4: 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 (e.g. 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, (e.g. 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[3]:

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
- Vasectomised partner
- True sexual abstinence, defined as refraining from heterosexual intercourse during the entire period of risk associated with the study treatments. 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 8.4.5. 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.

10.5 Appendix 5: List of Abbreviations and Definitions of Terms

Abbreviation	Definition
8OH-dG	8 hydroxy 2 deoxyguanosine
A1M	Alpha-1-microglobulin
ADA	Anti-drug antibodies
AE	Adverse Event
AKI	Acute kidney injury
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
ATC	Anatomical Therapeutic Chemical
AUC	Area under the plasma concentration time curve
AUCτ	Area under the curve from 0 to t, where t is the last measurable sample
BP	Blood pressure
CA	Competent Authority
CKD	Chronic kidney disease
CL	Total systemic clearance
C_{max}	Maximum (peak) concentration observed
CO	Cardiac output
CPB	Cardiopulmonary by-pass
C_{trough}	Lowest concentration reached by a drug before the next dose is administered
CrCl	Creatinine clearance
CRP	C-reactive protein
CSP	Clinical Study Protocol
CSR	Clinical Study Report
CTCAE	Common Terminology Criteria for Adverse Events
CV	Coefficient of variation
CVC	Central venous catheter
CVP	Central venous pressure
ECG	Electrocardiogram
eCRF	electronic Case Report Form
ELISA	Enzyme linked immunosorbent assay
FAS	Full Analysis Set
FDA	U.S. Food and Drug Administration
FSH	Follicle stimulating hormone
GCP	Good Clinical Practice
GFR	Glomerular filtration rate
CKD-EPI	Chronic Kidney Disease-Epidemiology Collaboration
GLP	Good Laboratory Practice

Abbreviation	Definition
GMP	Good Manufacturing Practice
h	hour(s)
Hb	Hemoglobin
IB	Investigator's Brochure
ICF	Informed consent form
ICH	International conference on harmonization
ICU	Intensive care unit
IEC	Independent ethics committee
IGFBP7	Insulin like growth factor binding protein 7
IL	Interleukin
IMP	Investigational Medicinal Product
INR	International normalised ratio
IRR	Infusion related reaction (Injury poisoning and procedural complications)
ISR	Infusion site reactions (General disorders and administration site conditions)
IV	Intravenous
KDIGO	a global nonprofit organization developing and implementing evidence-based clinical practice guidelines in kidney disease
KIM	Kidney injury marker
L-FAPB	Fatty acid-binding protein
LLOQ	Lower limit of quantification
MAD	Multiple ascending dose
MedDRA	Medical dictionary for regulatory activities
NCA	Non-compartmental analysis
NGAL	Neutrophil gelatinase associated lipocalin
NT-proBNP	N-terminal pro b-type natriuretic peptide
PA	Pulmonary artery
PK	Pharmacokinetic
PT	Preferred term
PTR	Peak to trough ratio
PV	Pharmacovigilance
QA	Quality assurance
$R_{ac\ (AUC)}$	Accumulation ratio calculated from AUCτ,ss and AUCτ after single dosing
ROS	Reactive oxygen species
RRT	Renal replacement therapy
SAD	Single ascending dose
SAE	Serious adverse event
SAP	Statistical analysis plan

Abbreviation	Definition
SCr	Serum Creatinine
SD	Standard deviation
SDV	Source data verification
SOC	System organ class
SUSAR	Suspected unexpected serious adverse reaction
SvO2	mixed venous saturation
TEAE	Treatment emergent adverse event
TIMP2	Tissue inhibitor of metalloproteinases 2
$t_{½}$	Elimination half-life
ULN	Upper limit of normal
WHO	World Health Organisation

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