AM-Pharma

AP-recAP-AKI-02-01

A Randomized, Double-Blind, Placebo-Controlled, Four-Arm, Parallel-Group, Proof of Concept, and Dose-Finding Adaptive Phase 2a/2b Study to Investigate the Safety, Tolerability and Efficacy and Effect on Quality of Life of Human Recombinant Alkaline Phosphatase in the Treatment of Patients With Sepsis-Associated Acute Kidney Injury

27SEP2017

Statistical Analysis Plan

Final Version 3.1

Prepared by:

PPD
Collette Letham, Biostatistician
PPD Global Biostatistics and Programming,
Fleming House 1,
Strathclyde Business Park,
Phoenix Crescent,
Bellshill,
Scotland
ML3 4NJ

Updated by:

Gillian Hopton, Biostatistician
PPD Global Biostatistics and Programming
Granta Park
Great Abington
Cambridge
CB21 6GQ

AM-Pharma

AP-recAP-AKI-02-01

A Randomized, Double-Blind, Placebo-Controlled, Four-Arm, Parallel-Group, Proof of Concept, and Dose-Finding Adaptive Phase 2a/2b Study to Investigate the Safety, Tolerability and Efficacy and Effect on Quality of Life of Human Recombinant Alkaline Phosphatase in the Treatment of Patients With Sepsis-Associated Acute Kidney Injury

27SEP2017

Statistical Analysis Plan

Final Version 3.1

Issued by: _____ Date: ___/___/___
Eszter Pulay
Lead Biostatistician, Biostatistics
PPD

Reviewed by: _____ Date: ___/___/___
Natalie Compton
Biostatistics Project Lead, Biostatistics
PPD

Upon review of this document, including table, listing, and figure shells, the undersigned approves the statistical analysis plan. The analysis methods and data presentation are acceptable, and the table, listing, and figure production can begin.

Approved by: _____ Date: ___/___/___
Jacques Arend
VP Clinical Development & CMO
AM-Pharma

TABLE OF CONTENTS

LIST OF ABBREVIATIONS VI

1. INTRODUCTION 1

2. OBJECTIVES 2

2.1. PRIMARY OBJECTIVES 2
2.2. SECONDARY OBJECTIVES 2
2.3. OTHER OBJECTIVES 2

3. INVESTIGATIONAL PLAN 3

3.1. OVERALL STUDY DESIGN AND PLAN 3
3.2. STUDY ENDPOINTS 4
3.2.1. Primary Endpoint 4
3.2.2. Key Secondary Endpoint 4
3.2.3. Other Secondary Endpoints 4
3.2.4. Other Endpoints 5
3.3. TREATMENTS 6
3.4. DOSE ADJUSTMENT/MODIFICATIONS 7

4. GENERAL STATISTICAL CONSIDERATIONS 7

4.1. SAMPLE SIZE 11
4.2. RANDOMIZATION, STRATIFICATION, AND BLINDING 11
4.3. ANALYSIS SET 12
4.3.1. All Enrolled 12
4.3.2. Intent-to-Treat Combined 13
4.3.2.1. ITT Part 1 13
4.3.2.2. ITT Part 1 Interim 13
4.3.2.3. ITT Part 2 13
4.3.3. Per Protocol 13
4.3.3.1. PP Day 1 – 7 Combined 13
4.3.3.2. PP Day 1 – 7 Part 1 14
4.3.3.3. PP Day 1–7 Part 2 14
4.3.4. Safety 14
4.3.5. Pharmacokinetics (PK) 15
4.3.6. Iohexol 15

5. PATIENT DISPOSITION 15

5.1. DISPOSITION 15
5.2. SCREEN FAILURES 15
5.3. PROTOCOL DEVIATIONS 16

6. DEMOGRAPHICS AND BASELINE CHARACTERISTICS 16

6.1. DEMOGRAPHICS 16
6.2. BASELINE DISEASE CHARACTERISTICS 16
6.3. MEDICAL HISTORY 17
6.4. INCLUSION AND EXCLUSION CRITERIA 17

7. TREATMENTS AND MEDICATIONS 18

7.1. CONCOMITANT MEDICATIONS 18
7.2. STUDY TREATMENTS 18
7.2.1. Extent of Exposure 18

8. EFFICACY ANALYSIS 19

8.1. PRIMARY EFFICACY ENDPOINT 19
8.1.1. Primary Analysis 21
8.1.2. Adjudication Committee 22
8.1.3. Assumption Testing 23
8.1.4. Sensitivity Analyses 23
8.1.5. Supportive Analysis for the Primary Efficacy Endpoint 24
8.2. SECONDARY EFFICACY ENDPOINT 25
8.2.1. Key Secondary Efficacy Endpoint 25
8.2.2. Other Secondary Efficacy Endpoints 26
8.2.2.1. Renal 27
8.2.2.2. Other Organs 30
8.2.2.3. Biomarkers 33
8.3. OTHER EFFICACY ENDPOINTS 34

9. SAFETY ANALYSIS 39

9.1. ADVERSE EVENTS 39
9.1.1. Incidence of Adverse Events 39
9.1.2. Relationship of Adverse Events to Study Drug 39
9.1.3. Severity of Adverse Event 40
9.1.4. Serious Adverse Events 41
9.1.5. Adverse Events Leading to Treatment Discontinuation 41
9.1.6. Death 41
9.2. LABORATORY EVALUATIONS 41
9.2.1. Hematology 42
9.2.2. Clinical Chemistry 42
9.2.3. Urinalysis 42
9.2.4. Pregnancy 42
9.3. VITAL SIGN MEASUREMENTS 43
9.4. PHYSICAL EXAMINATION 43
9.5. ELECTROCARDIOGRAM 44

10. PHARMACOKINETICS AND PHARMACODYNAMICS 44

11. INTERIM ANALYSIS 44

12. CHANGES IN THE PLANNED ANALYSIS 46

13. CHANGE HISTORY 47

14. REFERENCES 51

15. APPENDICES 52

15.1. SCHEDULE OF STUDY PROCEDURES 52
15.2. SCHEDULE OF DMC REVIEWS 58
15.3. WEIGHT RANGES AND PRE-CALCULATED CORRESPONDING VOLUMES 59
15.4. GFR FORMULA 60

LIST OF TABLES

TABLE 4-1 ACTUAL TREATMENT DOSE ASSIGNMENT 9
TABLE 4-2 SERUM CREATININE AND CRP LABORATORY CONVERSIONS 11
TABLE 8-1 EXAMPLE OF DERIVATION OF RRT-FREE DAYS AND DAYS ON RTT 28
TABLE 8-2 NORMAL RANGES FOR LUNG FUNCTION PARAMETERS 31
TABLE 12-1 SUMMARY OF CHANGES FROM PLANNED ANALYSIS AND REASON 47
TABLE 15-1 SCHEDULE OF ASSESSMENTS 52
TABLE 15-2 SCHEDULE OF DMC REVIEWS 58
TABLE 15-3 WEIGHT RANGES AND PRE-CALCULATED CORRESPONDING VOLUMES 59

List of Abbreviations

AE	adverse event
AKI	acute kidney injury
AKIN	Acute Kidney Injury Network
ANOVA	analysis of variance
AP	alkaline phosphatase
APACHE	acute physiology and chronic health evaluation
ALT	alanine aminotransferase
AST	aspartate aminotransferase
AUC_{1-7}	area under the time-corrected endogenous creatinine clearance curve from Day 1 to Day 7
BiAP	bovine intestinal alkaline phosphatase
BMI	body mass index
BUN	blood urea nitrogen
CI	confidence interval
CKD	chronic kidney disease
CKD-EPI	chronic kidney disease epidemiology collaboration
CMH	Cochran-Mantel-Haenzel
CRP	C-reactive protein
DMC	data monitoring committee
ECG	electrocardiogram
eCRF	electronic case report form
eGFR	estimated glomerular filtration rate
EQ-5D	EuroQol-5D
FeNa	fractional excretion of sodium
FE Urea	fractional excretion of urea
GFR	glomerular filtration rate
GGT	gamma-glutamyl transpeptidase
GST-alpha	alpha-glutathione s-transferase
hCG	human chorionic gonadotropin
ICH	International Conference on Harmonisation
ICU	intensive care unit
IgE	immunoglobulin E
IgG	immunoglobulin G
IL-6	Interleukin 6
IL-18	Interleukin 18
ITT	intent-to-treat
IV	intravenous
IVRS	interactive voice response system
KIM-1	Kidney injury molecule-1
LBP	lipopolysaccharide-binding protein
LDH	lactate dehydrogenase
LOCF	last observation carried forward
MedDRA	Medical Dictionary for Regulatory Activities
PAH	Para-aminohippuric

P/F ratio	fraction PaO_2/FiO_2 (Carrico index)
PD	Protocol deviations
PEEP	positive end expiratory pressure
PK	pharmacokinetic
PP	per protocol
PT	preferred term
PVG	pharmacovigilance
recAP	recombinant human AP
RRT	renal replacement therapy
SA-AKI	sepsis-associated acute kidney injury
SAE	serious adverse event
SAP	statistical analysis plan
SOC	system organ class
SOFA	system organ failure assessment
TEAE	treatment emergent adverse events
WHO	world health organization

1. Introduction

This statistical analysis plan (SAP) describes the planned statistical analysis for AP-recAP-AKI-02-01 study. It is based on the Protocol AP-recAP-AKI-02-01 Version 3.0, including Amendment 2 dated 3rd February 2016.

Sepsis-associated acute kidney injury (SA-AKI) is a serious condition with a mortality rate of up to 70%, while patients surviving an episode of acute kidney injury (AKI) are at risk of developing chronic kidney disease (CKD) (Oppert et al 2008; Chawla et al 2011, Vaara et al 2012).

As there are no guidelines for the development of drugs for the indication SA-AKI the proposed design of this study was determined to be optimal by a group of leading global experts in AKI and sepsis, and subsequently was discussed (and agreed) with European and United States regulatory agencies.

The study has been set up with an adaptive study design including 2 parts, with dose selection based on an interim analysis after all patients in Part 1 have completed the first 7 days of the study, unless the patient was randomized but died or discontinued prior to completing 7 days. The 3 doses proposed in Part 1 are selected based on a combination of information from previous clinical studies conducted with bovine intestinal alkaline phosphatase (BiAP), and pre-clinical animal models and pharmacokinetic (PK) modeling and simulation in a Phase I healthy volunteer study with recombinant human AP (recAP). Assuming comparable safety profiles, the most optimal dose will be selected at the interim analysis by an independent data monitoring committee (DMC). The primary endpoint, creatinine clearance, was chosen because as a continuous variable it is sensitive for detecting relatively small treatment effect differences of recAP versus placebo, as well as determination of effect size differences between the different dosages. Incidence of dialysis, considered to be a relevant clinical endpoint for Phase 3 pivotal studies, was chosen as the key secondary endpoint. In Part 1 PK samples will be taken to assess the pharmacological properties of recAP in patients, in addition to the information previously derived from the healthy volunteers.

Currently there is no treatment available for SA-AKI; hence, recAP is considered as add-on therapy and with that the use of a placebo arm is fully justified.

Following patients up to a period of 90 days allows for assessing potential disease-modifying characteristics of recAP in kidney function (occurrence or worsening of CKD).

2. Objectives

2.1. Primary Objectives

The primary objectives of the study are as follows:

- To investigate the effect of recAP on renal function and related clinical parameters in patients with SA-AKI.
- To determine the therapeutic dose(s) of recAP to support the pivotal Phase 3 program.

2.2. Secondary Objectives

The secondary objectives are as follows:

- To investigate the safety and tolerability of recAP in patients with SA-AKI.
- To investigate the PK of recAP in a subset (Part 1) of patients with SA-AKI.
- To investigate the immunogenic potential of recAP in patients with SA-AKI.
- To investigate the effect on quality of life (using the EuroQol, EQ-5D).

2.3. Other Objectives

To determine whether specific patient groups benefit most from recAP treatment and whether patient groups that are non-responders can be identified. The identification of such groups will be based on:

- Baseline characteristics, including:
  - Baseline kidney function marker (fractional excretion of sodium [FeNa], fractional excretion of urea [FE Urea])
  - Baseline tubular injury biomarkers (kidney injury molecule-1 [KIM-1], interleukin 18 [IL-18], alpha-glutathione s-transferase [GST-alpha])
  - Baseline biomarkers for systemic inflammation (IL-6, C-reactive protein [CRP], lipopolysaccharide-binding protein [LBP])
  - Baseline glomerular filtration rate (eGFR by chronic kidney disease epidemiology collaboration [CKD-EPI])
  - Baseline APACHE II score (<25, ≥ 25)
- Timing from first diagnosis of SA-AKI to start of recAP treatment, in time intervals (0 to < 6 hours, 6 to < 12 hours, 12 to < 18 hours, 18 to < 24 hours, ≥24 hours).
- Baseline Acute Kidney Injury Network (AKIN) stage (stage 1, stage 2, stage 3).

3. Investigational Plan

3.1. Overall Study Design and Plan

This is a randomized, double-blind, placebo-controlled, 4-arm, parallel-group, proof-of-concept, and dose-finding adaptive Phase 2a/2b study.

Approximately 290 patients with SA-AKI will be enrolled in the study. The study involves 2 parts (Part 1, Part 2) with an interim analysis between the parts, with some recruitment during this interim analysis. Of the 290 planned patients, at least 120 patients will enroll in Part 1 and 170 patients will enroll in Part 2. Patients enrolled during Part 1 will be randomly assigned to receive, by 1-hour intravenous (IV) infusion, either placebo (n1 = 30) or 1 of 3 different doses of recAP (n1 = 30 in each dosing arm; i.e., 0.4 mg/kg [250 U/kg], or 0.8 mg/kg [500 U/kg], or 1.6 mg/kg [1000 U/kg]) using a 1:1:1:1 allocation ratio. Patients will receive study drug once daily for 3 days (Days 1, 2, and 3). The interim analysis on the primary endpoint will be performed after 120 patients have completed the first 7 days in Part 1 (allowing for patients that were randomized but discontinued/died prior to 7 days) and all relevant data have been monitored to select the dose to be administered in Part 2. The dose chosen will be the most optimal dose of recAP on the primary endpoint in Part 1, provided there are no safety issues with that dose as judged by the DMC. While the intention is to choose the optimal recAP dose based on the primary efficacy endpoint results for Part 1, it is possible that this dosing group will have adverse safety issues. In this case, the next "best" dose with supportive safety data would be chosen for Part 2.

In Part 2, patients will be randomly assigned to receive either placebo ($n_2 = 85$) or the dose of recAP ($n_2 = 85$) selected during the interim analysis.

Each part involves the following schedule of events: potential patients who have been admitted to the intensive care unit (ICU)/Intermediate care will undergo a pre-screening, will provide informed consent, and will undergo screening assessments to determine eligibility. As soon as possible when inclusion and exclusion criteria are met, and after confirmation of continuing (i.e., not resolving) AKI by a fluid-corrected serum creatinine assessment or urine output, eligible patients will be randomly assigned to a treatment group (Baseline), undergo baseline determinations, and start treatment with study drug (Day 1). Treatment must be administered within 24 hours, at the latest, after SA-AKI is first diagnosed and within 96 hours from sepsis first diagnosis. Table 15-1 Schedule of Assessments

Assessment	**Screening (≤ 24 h)**	**Baseline (≤ 24 h)**	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7 (± 1 day)**	**Day 14 (± 2 days)**	**Day 21 (± 3 days)**	**Day 28 (± 3 days)**	**Day 60 (± 5 days)**	**Day 90 (± 10 days)**
AKI diagnosis[a] (pre-screening) Record site of infection and pathogen	X													
Inclusion and exclusion criteria	X	X[b]												
Informed consent	X													
Medical history	X													
Demographics	X													
Child-Pugh score[c]	X													
Recent hematology and clinical chemistry results, if available	X													
Recent microbial test results, if available	X													
Pregnancy test (urine or blood)[d]	X													
Local laboratory confirmatory serum creatinine sample[e], or confirmatory assessment of continuation of decreased urine output	X													

Assessment	**Screening (≤ 24 h)**	**Baseline (≤ 24 h)**	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7 (± 1 day)**	**Day 14 (± 2 days)**	**Day 21 (± 3 days)**	**Day 28 (± 3 days)**	**Day 60 (± 5 days)**	**Day 90 (± 10 days)**
Randomization[f]		X												
Vital signs (BP, HR, OS, RR, T)[g]		X	X[h]	X[h]	X[h]	X	X	X	X	X	X	X		
Physical examination		X	X	X	X	X	X	X	X	X	X	X		
APACHE II score		X												
SAPS-2 score		X												
SOFA score[i]		X	X	X	X	X	X	X	X	X	X	X		
EQ-5D**Error! Reference source not found.**		X												X
Alkaline phosphatase		X												
Time from first diagnosis of SA-AKI to start of recAP treatment		X												
Treatment			X	X[k]	X[k]									
Arterial partial pressure of O_2 (in ICU or intermediate care unit only) for mechanically ventilated patients		X	X	X	X	X	X	X	X	X	X	X		
Blood: serum creatinine and BUN[l]		X[e]	X	X	X	X	X	X	X	X	X	X	X	X
Urine (6 ± 1 h collection) creatinine, BUN[m]		X[n]	X	X	X	X	X	X	X	X	X	X		
Volume of urine[o]		X[p]	X	X	X	X	X	X	X	X	X	X		

Assessment	**Screening (≤ 24 h)**	**Baseline (≤ 24 h)**	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7 (± 1 day)**	**Day 14 (± 2 days)**	**Day 21 (± 3 days)**	**Day 28 (± 3 days)**	**Day 60 (± 5 days)**	**Day 90 (± 10 days)**
Pharmacokinetics[q]			X	X	X	X	X	X	X					
ECG (12-lead)[r]		X			X					X				
Hematology (Hgb, Hct, leukocytes, diff leukocytes, erythrocytes, thrombocytes, and APTT)[d]		X	X		X		X		X	X	X	X		
Clinical chemistry (CRP, ALT, AST, GGT, urea, LDH, creatinine, bilirubin, CPK, total protein, albumin, glucose, sodium, potassium, chloride, bicarbonate, and lactate)[d]		X	X		X		X		X	X	X	X		
Biomarkers for systemic inflammation (e.g., IL-6, CRP, LBP)[s]		X	X	X	X	X	X	X	X	X	X	X		
Serology (IgG, IgE, and total immunoglobulin)[s]		X								X		X		
Anti-drug antibodies		X								X		X	X[t]	X[t]

Assessment	Screening (≤ 24 h)	Baseline (≤ 24 h)	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7 (± 1 day)	Day 14 (± 2 days)	Day 21 (± 3 days)	Day 28 (± 3 days)	Day 60 (± 5 days)	Day 90 (± 10 days)
Tubular injury biomarkers (e.g., KIM-1, IL-18, GST-alpha) in urine[s]		X	X	X	X	X	X	X	X	X	X	X		
Kidney function markers (urine creatinine, BUN/urea clearance, fractional excretion of urea and urine output)[s]		X	X	X	X	X	X	X	X	X	X	X		
Kidney function markers (sodium and fractional excretion of sodium)[s]		X	X	X	X	X	X	X	X					
Kidney function markers (serum creatinine and proteinuria)[s]		X	X	X	X	X	X	X	X	X	X	X	X	X
Concomitant medication	X[u]	X	X	X	X	X	X	X	X	X	X	X		
Patient on RRT, and start or stop date			X	X	X	X	X	X	X	X	X	X		
Need for dialysis dependency													X	X
Name, start or stop date, and dose of vasopressor and inotropic therapy[v]		X	X	X	X	X	X	X	X	X	X	X		

Assessment	Screening (≤ 24 h)	Baseline (≤ 24 h)	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7 (± 1 day)	Day 14 (± 2 days)	Day 21 (± 3 days)	Day 28 (± 3 days)	Day 60 (± 5 days)	Day 90 (± 10 days)
Mechanical ventilation and lung function[w] (start or stop date, FiO_2, PEEP, tidal volume, P/F ratio), ventilated patients only		X	X	X	X	X	X	X	X	X	X	X		
Adverse events	X	X	X											
Mortality	X	X	X											
Discharge from ICU or intermediate care unit / admission or discharge from hospital[Error! Reference source not found.]	X	X	X											

Abbreviations: AKI = acute kidney injury; ALT = alanine aminotransferase; APACHE = acute physiology and chronic health evaluation; APTT = activated partial thromboplastin time; AST = aspartate aminotransferase; BP = blood pressure; BUN = blood urea nitrogen; CPK = creatine phosphokinase; CRP = c-reactive protein; diff = differential; ECG = electrocardiogram; FiO2 = fraction of inspired oxygen; GGT = gamma-glutamyl transpeptidase; GST-alpha = alpha-glutathione s-transferase; h = hour; Hct = hematocrit; Hgb = hemoglobin; HR = heart rate; ICU = intensive care unit; IgE = immunoglobulin E; IgG = immunoglobulin G; IL-6 = interleukin-6; IL-18 = interleukin-18; KIM-1 = kidney injury molecule-1; LBP = lipopolysaccharide binding protein; LDH = lactate dehydrogenase; OS = oxygen saturation; PEEP = positive end expiratory pressure; P/F ratio = fraction PaO_2/FiO_2; RR = respiratory rate; RRT = renal replacement therapy; SAPS-2 = simplified acute physiology score 2; SOFA = sequential organ failure assessment; T = temperature.

a. The AKI diagnosis can be made according to one of the AKIN serum creatinine criteria (absolute or relative increase, see inclusion criteria **Error! Reference source not found.** and **Error! Reference source not found.**), or according to the AKIN urine output criteria (urinary output < 0.5 mL/kg/h for > 6 hours, see inclusion criterion **Error! Reference source not found.**), the oliguria or anuria should still meet the AKIN urine output criteria prior to randomization and study drug administration.

b. Confirmatory.

c. Only for patients with liver disease.

d. Local laboratory.

e. See flowchart (Section **Error! Reference source not found.**, **Error! Reference source not found.**) for options and preference for reference serum creatinine value. The reference creatinine value is the serum creatinine value according to the following order of preference: 1) lowest value within 3 months of the hospital admission. If not

available, 2) at hospital admission. If not available, 3) at ICU or intermediate care unit admission. If not available, 4) lowest value between 3 and 12 months prior to hospital admission.

f. When the diagnosis of AKI is made according to one of the AKIN serum creatinine criteria (absolute or relative increase, see inclusion criteria **Error! Reference source not found.** and **Error! Reference source not found.**), patients will be eligible for the study and can be randomly assigned when the volume-corrected serum creatinine sample, taken at screening confirms the continuation of AKI according to the AKIN criteria for serum creatinine. When the AKI diagnosis was made according to the AKIN urine output criteria (urinary output < 0.5 mL/kg/h for > 6 hours, see inclusion criterion **Error! Reference source not found.**), the oliguria or anuria should still meet the AKIN urine output criteria prior to randomization and study drug administration.

g. Vital signs in ambulant patients will be obtained with the patient in a sitting position and after 5 minutes rest. Blood pressure will be monitored non-invasively. In patients who already have an arterial line placed as part of standard or care, readings from invasive blood pressure monitoring are to be recorded.

h. Additionally, vital signs (excluding temperature) will also be monitored during study drug infusion on all treatment days at the following times: a) immediately before the administration of the study drug, b) within 5 minutes of the start of the study drug infusion, c) 30 minutes after the start of the study drug infusion, d) immediately after the completion of the administration of the study drug, which includes post-dose saline flushing, e) 30 and 60 minutes after completion of study drug administration, f) 2 hours, 3 hours, 4 hours, and 5 hours after completion of study drug administration (Day 1 only).

i. SOFA score to be obtained on each visit day as long as the patient is in the ICU or intermediate care unit, and at discharge from ICU or intermediate care unit .

j. EQ-5D will be performed at baseline, at discharge from the ICU or intermediate care unit, and at the Day 90 visit. In case the patient is unconscious, EQ-5D questionnaire will be completed by a next of kin.

k. At 24 ± 1 hour after the previous drug administration.

l. Creatinine and BUN will be measured by a central laboratory. At Days 60 and 90, only serum creatinine will be measured. When patients have a Foley catheter, serum creatinine samples should be collected prior to and immediately after each urine collection for at least up to Day 7. If the patient is discharged from the ICU or intermediate care unit , the Foley catheter might be removed. In this case, a patient might urinate spontaneously and all efforts should be undertaken to start collecting urine produced from this time point onward. Approximately 6 hours later (exact duration needs to be recorded) the patient might urinate again and this urine will be used for analysis, and a blood sample will be drawn at this time too. The urine volume produced over approximately 6 hours will be entered in the eCRF.

m. Urine creatinine and urea will be measured by a central laboratory. The central laboratory will calculate blood urea nitrogen (BUN) clearance at all visits from Day 1 to Day 7, inclusive, and on subsequent visit days if reliable urine collection is possible. Urine will be collected within a 6 ± 1 hour period at all visits from Day 1 to Day 28, only if reliable urine collection is possible (i.e., patient remains in the ICU or intermediate care unit or hospital).

n. These assessments will be performed before treatment if possible. Treatment should not be delayed because of these assessments.

o. Urine volume collection in a 6 ± 1 hour collection period, only if reliable urine collection is possible (i.e., patient remains in the ICU or intermediate care unit or hospital). The volume should be corrected to account for the volume of samples previously taken from the total urine initially collected.

p. Only when possible within the 24-hour time window from first AKI diagnosis to treatment.

q. Assays will be performed by a central reference laboratory. See Section **Error! Reference source not found.** for sampling details.

r. A 12-lead ECG with at least 30-second rhythm strip will be recorded after the patient has rested supine or semi-recumbent for at least 5 minutes.

s. Central reference laboratory.

t. Samples taken on Days 60 and 90 will only be analyzed in case of a positive result on Day 28.

u. Verification that no concomitant medications that should be avoided are taken.

v. The actual stop date is collected for calculation of shock-free days. Only required when the patient is in the ICU or intermediate care unit.

w. Daily, as long as the patient requires mechanical ventilation. As appropriate, record start and stop dates and times of mechanical ventilation, including the settings required and the O_2 in the blood.

x. Actual admission dates and both planned and actual discharge dates from the ICU or intermediate care unit and hospital (planned meaning when decision is taken to discharge the patient, not necessarily being the same as the actual discharge date, e.g., because of lack of beds on the regular ward).

contains the schedule of all assessments throughout the study.

Due to potential unblinding it is not allowed to locally determine AP levels in the blood for 14 days (Visit day 14 included). From baseline up to Day 7, recAP PK data will be analyzed by a central laboratory.

3.2. Study Endpoints

3.2.1. Primary Endpoint

The primary endpoint is the area under the time-corrected endogenous creatinine clearance curve from Day 1 to Day 7 (AUC_{1-7}) calculated as the average of the standardized endogenous creatinine clearance values over the 7 days.

3.2.2. Key Secondary Endpoint

The key secondary endpoint is renal replacement therapy (RRT) incidence during the period Day 1 (after first treatment) to Day 28, inclusive.

3.2.3. Other Secondary Endpoints

Renal endpoints include the following:

- Volume of urine (daily from Day 1 to Day 7, inclusive, and on subsequent visit days if reliable 6 hour urine collection is possible) normalized per hour.
- Serum creatinine and blood urea nitrogen (BUN)/urea (daily from Day 1 to Day 7, inclusive, and on subsequent visit days)
- BUN/Urea clearance daily from Day 1 to Day 7 inclusive, and on subsequent visit days if reliable urine collection is possible.
- Peak value of serum creatinine and peak values of BUN/urea (during the period Day 1 to Day 7, inclusive)
- RRT-free days. An RRT-free day is defined as a day on which a patient did not receive any form of RRT.
- Total number of days on RRT (during the period Day 1 to Day 28, inclusive). A day on RRT is defined as a day on which a patient received any form of RRT (including RRT with interruptions) for any period of time on that day.
- Reasons for initiation of RRT (during the period Day 1 to Day 28, inclusive)

- Kidney function at Day 14, 21 and 28 as assessed by measured creatinine clearance if available, otherwise as assessed by estimated glomerular filtration rate (eGFR) (estimated by the CKD-EPI formula based on serum creatinine)
- Kidney function at Day 60 and Day 90 as assessed by eGFR (estimated by the CKD-EPI formula based on serum creatinine).
- Sustained loss of kidney function at Day 60 and Day 90, defined by eGFR < 60 mL/min (with eGFR estimated by the CKD-EPI formula based on serum creatinine).
- Incidence of dialysis dependency at Day 60 and Day 90.

Endpoints for organs other than renal function include the following:

- Liver enzymes (aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transpeptidase (GGT), lactate dehydrogenase (LDH), bilirubin but excluding alkaline phosphatase (AP)).
- Lung function as assessed by fraction PaO_2/FiO_2 (P/F ratio) Carrico index, positive end expiratory pressure (PEEP), and tidal volume in mechanically ventilated patients.
- Mechanical ventilator-free days (from Day 1 to Day 28, inclusive). A ventilator-free day is defined as a day on which a patient was not on ventilator (invasive or non-invasive mechanical ventilation).
- Time from start of first administration of study drug to being off- mechanical ventilator (from Day 1 to Day 28, inclusive) for those patients who are on mechanical ventilator at the start of this period.
- Shock-free days (during the period Day 1 to Day 28, inclusive). A patient is considered to be shock free if he or she is not on vasopressors or inotropic agents (including but not limited to noradrenaline, adrenaline, dobutamine, dopamine, vasopressin, or enoximone).
- Time from start of first administration of treatment to being shock free (from Day 1 to Day 28, inclusive) for those patients who are not shock free at the start of this period.
- System Organ Failure Assessment (SOFA) scores during ICU/Intermediate care stay.
- Number of dysfunctional organs as assessed by SOFA scores (from Baseline to Day 28, inclusive).
- Deaths during the 90-day study period (by recording date).

Biomarker endpoints include the following:

- Kidney function markers.
- Tubular injury biomarkers.
- Biomarkers for systemic inflammation.
- Pharmacokinetics of recAP in all 3 active treatment groups during Part 1 of the study.
- In addition to recAP PK concentration measurements, baseline (pre-dose) AP will be measured by activity (central laboratory).

3.2.4. Other Endpoints

Additional endpoints include the following:

- Composite endpoints – patients that meet, or do not meet, at least 1 of the following criteria:
  - Received RRT or died (prior to Day 28 [inclusive]).
  - eGFR < 60 mL/min (at Day 60, estimated by the CKD-EPI formula), or dialysis dependency, or died (prior to Day 60).

- eGFR < 60 mL/min (at Day 90, estimated by the CKD-EPI formula), or dialysis dependency, hospitalized for a new episode of AKI (at Day 90), or died (prior to Day 90).
- Serology as assessed by immunoglobulin G (IgG), immunoglobulin E (IgE), and total immunoglobulin.
- Safety parameters including (Serious) adverse events ((S)AEs), laboratory assessments (clinical chemistry, hematology, and urinalysis parameters not considered in the efficacy analysis), vital signs, and electrocardiogram (ECG) data.
- Quality of life, assessed by the EuroQol-5D (EQ-5D) questionnaire at baseline, ICU/Intermediate care discharge, and Day 90.
- Time from start of first administration of treatment to discharge from ICU/Intermediate care where discharge is defined as the time when the decision was made to transfer the patient (as opposed to the time of actual transfer).
- Total time in ICU/Intermediate care from the start of first administration of study drug (during the period Day 1 to Day 28 inclusive and during the period Day 1 to Day 90, inclusive) using the time of actual transfer
- Time from start of first administration of treatment to discharge from hospital where discharge is defined as the time when the decision was made to transfer the patient (as opposed to the time of actual transfer).
- Total time in hospital from the start of first administration of study drug (during the period Day 1 to Day 28, inclusive and during the period Day 1 to Day 90, inclusive) using the time of actual transfer.

3.3. Treatments

Study drug will be administered by 1-hour IV infusion as soon as possible on Day 1, and 24 ± 1 hours later on Days 2 and 3, by trained staff in the ICU/Intermediate care. A total of 50 mL will be infused at a constant rate of 50 mL/hour. At the start of each drug administration, the exact volume of recAP or placebo to be administered to each patient will be determined according to the patient's weight at screening. The volume of the placebo and the volume of the active doses of recAP are identical. The preferred route for study drug administration will be through a central line; if this is not possible, a peripheral line will be acceptable. Study drug will be administered separately from any concomitant drugs using a dedicated lumen of the catheter.

3.4. Dose Adjustment/Modifications

Infusion of the study drug may be temporarily interrupted or permanently discontinued for a variety of reasons, including an adverse event or equipment malfunction. Therefore, it is possible that the total study drug dose and volume planned for a patient may differ from the total dose and volume administered and any such patients will be identified on the study drug infusion listing (see Section 7.2).

4. General Statistical Considerations

Combined analysis of primary endpoint

As described in Section 8.1.1, the primary efficacy endpoint for this study is analyzed at both the interim analysis (based on all patients recruited in Part 1 up to that point) and at the end of the study (using all patients recruited after conclusion of Part 1, i.e. during Part 2). Patients recruited whilst the interim analysis is performed will be handled as follows:

- Those recruited to the optimal dose or placebo will form part of the Part 2 populations.
- Those recruited to the other doses will not be included in any efficacy analysis (except one sensitivity analysis on the primary endpoint), but their safety data will be analyzed.

The results from each analysis are then combined using an inverse normal method. This combined analysis of patients from Parts 1 and 2 rests on the assumption that patients recruited in each part of the study will belong to the same overall patient population. To check this assumption, data related to the baseline characteristics and demographics (for the selected optimal dose and placebo groups) will be repeated for Part 1 and Part 2. If any notable differences are observed, then the combined analysis will need to be interpreted with caution and further exploratory analysis will need to be conducted to investigate the possible causes.

The assumption of a consistent treatment effect across Parts 1 and 2 will be investigated by repeating the primary and key secondary efficacy analyses (for the optimal recAP dose and placebo treatment groups) for Part 1 and Part 2. Again, any notable differences will lead to the associated combined analysis being interpreted with caution and further exploratory analyses conducted to investigate the possible causes.

Combined analysis of secondary and 'other' endpoints

Efficacy endpoints will be summarized for all four treatment groups excluding patients recruited whilst the interim analysis is performed to treatment arms not selected for Part 2. Any analyses of the other efficacy endpoints will compare the optimal recAP dose and placebo treatment groups only. All safety data will be summarized for all four treatment

groups including patients recruited at any point in the study. Similarly, all data listings will include patients recruited at any point in the study.

See also Section 4.3 for definitions of the analysis sets used in the study, which also state which patients (i.e. recruited during Part 1, or recruited during Part 2) are included in each analysis set.

Display of data

Continuous variables will be summarized using descriptive statistics, including but not limited to the mean, standard deviation, median, lower quartile, upper quartile, minimum value, and maximum value. For the summary statistics of all numerical variables minimum and maximum will be displayed to the same level of precision as reported, unless otherwise specified. Mean, median, lower quartile and upper quartile will be displayed to one level of precision greater than the data collected. Standard deviation / standard error will be displayed to two levels of precision greater than the data collected. P-values will be rounded to three decimal places. If a p-value is less than 0.001 it will be reported as "<0.001." If a p-value is greater than 0.999 it will be reported as ">0.999".

Categorical variables will be summarized using frequency counts and percentages. When count data are presented, the percentage will be suppressed if the count is zero in order to draw attention to the non-zero counts. The denominator for all percentages will be the number of patients in that treatment within the analysis set of interest, unless otherwise specified. All percentages will be presented to one decimal place. Categorical summaries will display all categories available on the electronic case report form (eCRF). A row denoted "Missing" will be included in count tabulations only when information is missing for a categorical variable.

All data summaries will be provided by treatment arm.

All data collected across the 4 treatment groups throughout the study will be listed in data listings. Data will be displayed in all listings sorted by treatment group, patient ID and other variables as appropriate. Patients will be identified in the listings by the patient identification number concatenated with the investigator number.

In the case that there are no data available for a table, listing or figure, e.g. no serious adverse events (SAE) within the trial, an output should be created but state "There are no data to display for this [table/listing/figure]."

Definition of baseline

Unless otherwise specified, baseline will be defined as the last non-missing evaluation prior to the first infusion of study treatment. For the efficacy analyses which are based on

the ITT analysis set, any subjects who were not dosed will have their baseline defined as the last non-missing evaluation.

Study Time

Study day is defined as:

Assessment date - first infusion date of study treatment + 1.

Assessments will be performed during a total of 12 visits, at the following intervals: daily from Day 1 to Day 6; Day 7 + 1 day; weekly on Day 14 ± 2 days, Day 21 ± 3 days, and Day 28 ± 3 days; and follow-up assessments will be completed on Day 60 ± 5 days and Day 90 ± 10 days.

Method of assigning patients to received treatment group

Actual treatment received for a patient will be calculated as the average dose received over the three treatment days. The average dose received will be determined according to the treatment kit dispensed to the patient (per the materials/kit schedule) and the volume of infusion received as recorded on the treatment administration page of the eCRF.

For the safety and per protocol (PP) analyses, patients are analyzed according to their received treatment group. Using the average dose received over the three treatment days, each patient will be assigned to a received treatment group based on the dose windows defined in Table 4-1.

Table 4-1 Actual Treatment Dose Assignment

Average dose received	Received treatment group assignment
>0.0 – 0.62 mg/kg	recAP 0.4 mg/kg [250 U/kg]
>0.62 – 1.23 mg/kg	recAP 0.8 mg/kg [500 U/kg]
>1.23 mg/kg	recAP 1.6 mg/kg [1000 U/kg]

Please note that any patients who do not receive any dose of study drug will be excluded from the safety and PP analyses, and therefore do not need to be considered in the Table 4-1. Dose windows were constructed in line with the weight ranges and corresponding pre-calculating volumes displayed in Table 15-3. See Section 15.3 for full justification of these windows.

Statistical Analysis

All statistical tests will be two-sided and performed using a 5% significance level, displaying 95% two-sided confidence intervals (CIs), unless specifically stated otherwise. As the primary efficacy endpoint is analyzed at the interim analysis and at the end of the study, multiplicity will be addressed by using a combination test to combine the results (see Section 8.1.1). A hierarchical method will be employed to address any multiplicity arising from the analysis of the key secondary endpoint. In other words, the formal analysis of this endpoint will be performed only if a statistically significant result is obtained from the combination test analysis of the primary endpoint. All analyses performed on the other secondary endpoints are for exploratory purposes only; therefore, no further multiplicity adjustment is required.

All analyses will be undertaken using SAS® Version 9.2 (SAS Institute, Inc, Cary, North Carolina) or later.

Weight

Body weight (in kg or lb) will be measured or estimated at screening. Weight will be displayed in kg and derived from lb as:

$$Weight[kg] = \frac{Weight[lb]}{2.2046}$$

Temperature

Temperature (in °C or °F) will be measured from Day 1 – Day 28. Temperature will be displayed in °C and derived from °F as:

$$Temperature[°C] = \frac{Temperature[°F] - 32}{1.8}$$

Laboratory Data

For laboratory data that are recorded as values of '<*x*' and '>*x*' where *x* is any numeric value, the numeric part after the '>' and '<' (i.e. *x*) will be used for summarizing and in graphical presentations. In addition the numeric part will be used in the derivation of the quartile-based subgroup definitions.

In the tables where serum creatinine or CRP data are presented, if a subject has missing baseline central laboratory measured serum creatinine or CRP values, but has available baseline local laboratory measured values then the local laboratory values will be used to

reduce the number of missing baseline values in the analyses. This does not apply to the calculation of endogenous creatinine clearance. The following conversion rates will be used to convert the local lab measured value to mg/dL (if source unit is different):

Table 4-2 Serum Creatinine and CRP Laboratory Conversions

Parameter	Source Unit	Standard unit	Conversion rate
Serum creatinine	mg/L	mg/dL	0.1
	umol/L	mg/dL	0.0113122
CRP	mg/L	mg/dL	0.1
	nmol/L	mg/dL	0.0105

4.1. Sample Size

A sample size of $n_1 = 30$ patients per treatment group in Part 1 with an additional $n_2 = 85$ patients recruited to each of the optimal recAP dose and placebo treatment groups in Part 2 (for a total sample size of n = 290 patients) is planned. Recruitment will continue during the interim analysis, so the total number of patients will exceed 290. Custom programmed simulations were performed using SAS® Version 9.2 (SAS Institute, Inc, Cary, North Carolina) to determine power and type I error rate of the chosen sample size and design under a number of different dose response scenarios. Each scenario assumed a standard deviation of 49 mL/min for the primary endpoint (area under the time-corrected endogenous creatinine clearance curve from Day 1 to Day 7 [AUC_{1-7}]) with an assumed response of 60 mL/min for the placebo group, and between 60 mL/min (no treatment effect) and 79 mL/min (strong treatment effect) for the recAP dose groups.

Fifty-thousand simulations were performed to show that the 1-sided type I error rate is 2.4% (and hence is well controlled at the 1-sided 2.5% significance level). The power was defined as the probability of rejecting the null hypothesis (of no difference between treatment groups) when 1 or more recAP dose groups have an effective treatment effect, defined as a response of 69.5 mL/min. This was investigated across 7 scenarios with 10 000 simulations performed for each. In the most realistic scenarios (with strong treatment effects, i.e., responses of 79 mL/min for the medium and high recAP dose groups and a varying response of between 60 mL/min and 79 mL/min for the low-dose group) the chosen design achieved power of between 79% and 86%. This dropped to 66% to 67% for other scenarios where only the high recAP dose group had a strong treatment effect.

4.2. Randomization, Stratification, and Blinding

Patients will be randomly assigned to receive either placebo or 1 of 3 doses of recAP using a 1:1:1:1 allocation ratio. The randomization schedule is stratified by site. Once it has been

decided which is the most optimal dose of recAP on the primary endpoint to be administered in Part 2, the codes for the treatment groups corresponding to the 2 dropped doses will be discontinued, and treatment allocation will continue using the codes for the remaining treatments on the same schedule.

An interactive voice response system (IVRS) will be used to administer the randomization schedule. An independent PPD statistician generated a permuted block randomization schedule using SAS® Version 9.2 (SAS Institute, Inc, Cary, North Carolina) for IVRS, which will link sequential patient randomization numbers to treatment codes. Each patient will be assigned a randomization number which will be separate from the patient identification number. Once a randomization number has been allocated to a patient, it will not be assigned to another patient.

All persons involved in the study (including but not limited to the patient, site staff, AM-Pharma B.V. team members, and PPD blinded team members [i.e. those not involved with the interim analysis and DMC]) will be blinded to treatment assignment. The randomization schedule will be held by an independent PPD team at a different regional location and will not be revealed until all patients have completed the study and the database has been finalized for the end of the study, except for the unblinded interim analysis and DMC (see Section 11). To maintain the blind, the DMC and interim analyses will be conducted and delivered by an unblinded PPD Biostatistics team located at a different site to the blinded PPD biostatistics personnel involved in the study.

4.3. Analysis Set

In accordance with International Conference on Harmonisation (ICH) recommendations in guidelines E3 and E9, the following analysis sets will be used for the analyses.

4.3.1. All Enrolled

The all Enrolled Set includes all patients that have been assigned a patient number in either Part 1 or Part 2 of the study regardless of whether they were randomized or received study drug. Patients will be analyzed according to the treatment they were randomly assigned to, with a “Not randomly assigned” treatment group (screening failures) included if required.

4.3.2. Intent-to-Treat Combined

The Intent-to-Treat (ITT) Combined Set includes all patients who were randomly assigned to a study drug in either Part 1 or Part 2 of the study, excluding patients recruiting whilst the interim analysis is performed to treatment arms not selected for Part 2. This is the primary analysis set for the efficacy analyses, and patients will be analyzed according to the treatment to which they were randomly assigned.

4.3.2.1. ITT Part 1

The ITT Part 1 Set includes all patients who were randomly assigned to a study drug prior to the conclusion of Part 1 of the study excluding patients enrolled during the interim analysis on placebo and the selected optimal recAP dose group (these patients will be included in Part 2 of the study). This analysis set will be used to compare patients enrolled during the different parts of the study. Patients will be analyzed according to the treatment they were randomly assigned to.

4.3.2.2. ITT Part 1 Interim

ITT Part 1 interim set included all patients who were randomly assigned to a study drug prior to the conclusion of Part 1 of the study and were included in the interim analysis. Patients will be analyzed according to the treatment they were randomly assigned to.

4.3.2.3. ITT Part 2

The ITT Part 2 Set includes all patients who were randomly assigned to a study drug after the conclusion of Part 1 of the study and patients enrolled during the interim analysis on placebo and the selected optimal recAP dose group. This analysis set will be used to compare patients enrolled during the different parts of the study. Patients will be analyzed according to the treatment they were randomly assigned to.

4.3.3. Per Protocol

Analysis on the PP Set will be used as a supplement to the ITT analysis and will be performed for the primary efficacy and key secondary endpoint described as follows:

4.3.3.1. PP Day 1 – 7 Combined

The PP Day 1 – 7 Combined Set includes all patients who were randomly assigned to a study drug in either Part 1 or Part 2 of the study, had no significant protocol deviations (PDs) (e.g. non-adherence to inclusion/exclusion criteria with enrollment of the patient, or non-adherence to FDA regulations or ICH E6(R1) guidelines) and had complete data

(meaning no more than two missing day 1-7 creatinine clearance values) for the primary endpoint. The primary endpoint will be analyzed for the PP Day 1-7 Combined Set, with patients analyzed according to the treatment they received. See Section 10.2.2 of the protocol and the Significant Protocol Deviation Rules document for further information on the handling of PDs for this study. Significant PDs will be identified prior to study unblinding and summarized by the deviation categories specified above.

4.3.3.2. PP Day 1 – 7 Part 1

The PP Day 1 – 7 Part 1 Set includes all patients who were randomly assigned to a study drug prior to the conclusion of Part 1, excluding patients enrolled during the interim analysis on placebo and the selected optimal recAP dose group and are included in the ITT Part 1 Interim Set, had no significant protocol deviations (PDs) (e.g. non-adherence to inclusion/exclusion criteria with enrollment of the patient, or non-adherence to FDA regulations or ICH E6(R1) guidelines) and had complete data (meaning no more than two missing day 1-7 creatinine clearance values) for the primary endpoint. The primary endpoint will be analyzed for the PP Day 1-7 Part 1 Set, with patients analyzed according to the treatment they received. See Section 10.2.2 of the protocol and the Significant Protocol Deviation Rules document for further information on the handling of PDs for this study. Significant PDs will be identified prior to study unblinding and summarized by the deviation categories specified above.

4.3.3.3. PP Day 1–7 Part 2

The PP Day 1–7 Part 2 Set includes all patients who were randomly assigned to a study drug after the conclusion of Part 1 of the study, including patients enrolled during the interim analysis on placebo and the selected optimal recAP dose group, had no significant PDs (e.g. non-adherence to inclusion/exclusion criteria with enrollment of the patient, or non-adherence to FDA regulations or ICH E6(R1) guidelines) and had complete data (meaning no more than two missing day 1-7 creatinine clearance values) for the primary endpoint. The primary endpoint will be analyzed for the PP Day 1-7 Part 2 Set, with patients analyzed according to the treatment they received. See Section 10.2.2 of the protocol and the Significant Protocol Deviation Rules document for further information on the handling of PDs for this study. Significant PDs will be identified prior to study unblinding and summarized by the deviation categories specified above.

4.3.4. Safety

The Safety Set includes all patients who were randomly assigned and received a dose of study drug. All safety analyses will be based on the Safety Set, with patients analyzed according to the treatment they received.

4.3.5. Pharmacokinetics (PK)

The PK Set includes all patients who were randomly assigned and received at least one treatment during Part 1 of the study. The PK analyses will be based on this set, with patients analyzed according to the treatment they received.

4.3.6. Iohexol

The Iohexol Set includes all patients who were administered iohexol.

5. Patient Disposition

5.1. Disposition

Patient disposition will be summarized for the all Enrolled Set. A disposition of patients includes the number and percentage of patients for the following categories: patients who were not randomly assigned to treatment, patients in the ITT Combined Set, patients treated (Safety Set), patients in the PP Day 1-7 Combined Set, patients in the PP Day 1-7 Part 1 Analysis Set, patients in the ITT Part 1 Set, patients in the ITT Part 1 Interim Set, patients in the ITT Part 2 Set, patients in the PP Day 1-7 Part 2 Set, patients in the PK Set and patients in the Iohexol Set. The number and percentage of patients who completed the study and patients who discontinued from the study will be summarized for the ITT Combined Set, the ITT Part 1 Set, ITT Part 1 Interim Set and the ITT Part 2 Set. The percentages will be based on the number of patients in each analysis set.

The reasons for study discontinuation will also be summarized in this table. The reason for study discontinuation includes the following: Adverse Event, Lab abnormality, Patient Withdrew consent, Investigator Decision, Sponsor termination of study, Protocol Non-Compliance or violation, Death and Other. The percentages will be based on the number of patients who discontinued from the study.

The disposition table will be repeated by site for the all Enrolled Set.

Patient disposition data will be presented in a listing.

5.2. Screen Failures

All patients who are screen failures will be presented in a listing for the all Enrolled Set, together with the reason for failing screening.

5.3. Protocol Deviations

All PDs will be recorded throughout the study (recorded on the clinical trial management system), and reviewed on an ongoing basis. Significant PDs will be identified according to the Significant PD Rules for the study, and presented in a listing for the all Enrolled Set.

6. Demographics and Baseline Characteristics

6.1. Demographics

A summary of demographics and baseline information will be presented for the ITT Combined Set, ITT Part 1 Set and ITT Part 2 Set. The demographic characteristics consist of age (years), sex and race. Baseline weight can be recorded on the eCRF at screening in either kg or lb, weight in lb will be converted to kg using the calculation in Section 4. Baseline weight (kg), baseline height (cm), and body mass index (BMI) (kg/m^2) will be included in the summary.

A patient's age in years will be calculated using the date of the informed consent and date of birth. Age (years) and baseline weight (kg) will be summarized using descriptive statistics. The number and percentage of patients by age category (<55, ≥55 - < 70, ≥70), sex (Male, Female) and race (Caucasian, Black, Asian, Other and Not Collectable), will also be reported. Not Collectable category is available as race is not allowed to be collected in certain countries.

Patient demographic and baseline characteristics will be presented in a listing based on the ITT Combined Set.

6.2. Baseline Disease Characteristics

The distribution of patients across treatment groups will be presented for the following characteristics. This will be in the form of descriptive statistics for continuous data, or as the number and percentage of patients included in each category for categorical data. This summary will be presented for the ITT Combined Set, and also for the ITT Part 1 Set and ITT Part 2 Set to check the assumption that patients recruited in each part of the study will belong to the same overall patient population. This will include:

- Fractional excretion of sodium and fractional excretion of urea summarized by descriptive statistics;
- eGFR by CKD-EPI by descriptive statistics;
- SAPS-2 by descriptive statistics;
- SOFA score by descriptive statistics;

- Alkaline Phosphatase activity by descriptive statistics;
- The number and percentages of patients by mechanical ventilation at baseline categories (yes, no);
- The number and percentages of patients by infectious agent categories (yes, no);
- The number and percentages of patients by AKI stage (stage 1, stage 2, stage 3) according to the AKIN criteria;
- Heart rate by descriptive statistics;
- Systolic blood pressure by descriptive statistics;
- Diastolic blood pressure by descriptive statistics;
- The number and percentages of patients by body temperature categories (<36^{o}C, >=36 ^{o}C to <=38 ^{o}C and >38^{o}C);
- Urine output by descriptive statistics;
- Serum creatinine at baseline from the central laboratory data by descriptive statistics; values will be converted to mg/dL; if baseline central laboratory data is unavailable, but there is local laboratory measured serum creatinine available for the subject, then the baseline value obtained by the local laboratory will be used;
- Creatinine clearance (using the formula given in 8.1 to derive the measured value) by descriptive statistics;
- Acute physiology and chronic health evaluation (APACHE) II score by descriptive statistics and by frequency count (<25 vs. ≥25);
- The number and percentages of patients with vasopressor/inotropic therapy use at baseline (yes, no)

The baseline disease characteristics and microbes specified for infectious agents, for all patients in the ITT Combined Set will also be presented in a listing.

6.3. Medical History

Medical history is collected at screening and will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) Version 16.1 or later. The number and percentage of patients with any medical history will be summarized overall and for each system organ class (SOC) and preferred term (PT) for the ITT Combined Set. At each level of patient summarization, a patient is counted once.

Patient medical history data including specific details will be presented in a listing.

6.4. Inclusion and Exclusion Criteria

A listing, based on the all Enrolled Set, will be included displaying which protocol version/amendment each patient was recruited under, and whether the patient met and/or did not meet the inclusion and exclusion criteria.

7. Treatments and Medications

7.1. Concomitant Medications

Concomitant medications will be collected throughout the study and coded using the latest version of World Health Organization (WHO) Drug in use at the time of coding. The total number of concomitant medications and the number and percentages of patients with at least one concomitant medication will be summarized by treatment group. The number and percentages of all concomitant medications will be summarized by treatment group and listed by drug class and PT. At each level of patient summarization, a patient is counted once.

All summaries will be performed using the Safety Set.

Patient concomitant medication data including specific details will be presented in a listing.

7.2. Study Treatments

RecAP is supplied as a clear, colorless, pyrogen-free solution in 10 mL type 1 glass vials. Each vial contains 5 mL of recAP solution. Matching placebo is supplied as a clear, colorless, pyrogen-free solution in 10 mL type 1 glass vials. Each vial contains 5 mL of placebo solution.

Patient doses will be prepared from 4 vials in a combination of recAP drug product and recAP placebo. The content of the 4 vials will be used to fill an IV dosing syringe (50 mL) with an appropriate volume corresponding to the body weight of the patient, followed by addition of physiological saline solution to a total of 50 mL.

7.2.1. Extent of Exposure

The number and percentages of patients that received any dose on Day 1, Day 2 and Day 3 will be summarized by treatment group. The number and percentages of patients will also be presented by total number of doses received (1 dose, 2 doses, 3 doses) by treatment group. All summaries will be performed using the Safety Set. The number and percentage of patients who discontinued the study prior to receiving treatment on Day 1, Day 2 and Day 3 will also be included.

Study Drug Infusion information will be presented in the form of a listing for the Safety Set, including: was patient dosed (Yes, No); infusion start time; infusion end time; route of

administration (central line, peripheral line); volume of all 4 vials drawn into the 50 mL syringe (yes, no); volume from the 4 vials discarded from the syringe based on patients weight (mL); saline added to the 50 mL syringe (mL), was total volume of 50 mL syringe administered (yes, no), and, if no, reason why total volume was not administered; and total volume of study drug administered (mL).

8. Efficacy Analysis

A hierarchical method will be employed to address any multiplicity arising from the analysis of the key secondary endpoint. In other words, the formal analysis of this endpoint will be performed only if a statistically significant result is obtained from the combination test analysis of the primary endpoint. All analyses performed on the other secondary endpoints are for exploratory purposes only; therefore, no further multiplicity adjustment is required. The results of this hierarchical method will be presented indicating where statistically significant results are obtained.

To provide an overall summary of the efficacy analyses, a table will be produced containing the results and magnitude of difference between the optimal recAP dose and placebo treatment groups for certain key endpoints. This table will be based on the ITT Combined Set.

Supportive analysis will be undertaken on a selection of endpoints (AUC_{1-7} and RRT incidence from Day 1 to Day 28, inclusive) including specific group categories as factors to assess the consistency of treatment effects.

8.1. Primary Efficacy Endpoint

The primary endpoint will be calculated from standardized endogenous creatinine clearance measurements on Day 1 (first measurement after treatment) to Day 7, inclusive. Standardized endogenous creatinine clearance is assessed on each day during a 6 ± 1 hour period and calculated in mL/min as the mean creatinine clearance over the period, which is expected to be representative of the full 24 hours for that day.

An adjudication committee will review the standardized endogenous creatinine clearance data on a case-by-case basis to determine the values to be used in the analysis and for the derivation of AUC_{1-7} (see Section 8.1.2). The standardized endogenous creatinine clearance (mL/min) will be calculated by PPD Biostatistics using the following formula.

$$\frac{\text{Urine Creatinine (mg/dL)}}{\text{Serum Creatinine (mg/dL)}} \times \frac{\text{Total urine volume collected (mL)}}{\text{Stop date/time – Start date/time of urine collection (min)}}$$

- The urine creatinine (mg/dL) and serum creatinine (mg/dL) assessed by the central laboratory will be used to determine the standardized endogenous creatinine clearance.
- Per protocol, two blood samples are collected to evaluate the serum creatinine, one at the beginning of the urine sample collection and the other at the end of the urine sample collection. The average of these two serum creatinine measurements will be used in the formula above to assess the standardized endogenous creatinine clearance. In case there is only one serum creatinine sample available, then the serum creatinine concentration from that one sample will be used in the formula.
- The number of minutes from the start date/time to the stop date/time of urine sample collection and the total urine volume (mL) will be derived from the Urine Volume eCRF page. If the urine volume is recorded as 0 mL, then the standardized creatinine clearance will be 0 mL/min and will not be set to missing. If the urine volume is not recorded as missing but the volume is less than 30mL/24 hrs, then the standardized creatinine clearance will be set to 1 mL/min.

The adjudicated Part 2 data will be used to determine the AUC_{1-7} (primary endpoint), while the adjudicated Part 1 data will be used in a sensitivity analysis of the primary endpoint (as described in section 8.1.4). All data will be listed.

The primary endpoint is the area under the time-corrected endogenous creatinine clearance curve from Day 1 to Day 7 (AUC_{1-7}).

AUC_{1-7} is calculated as the average of the standardized endogenous creatinine clearance values over the 7 days. Specifically, denoting C_i as the standardized endogenous creatinine clearance on Day i, AUC_{1-7} is defined as:

$$\mathrm{AUC}_{1-7} = \frac{1}{7}\sum_{i=1}^{7} C_i$$

Any missing C_i values will be handled by linear interpolation where possible, otherwise they will be imputed by last observation carried forward (LOCF). When there are no preceding post-baseline measurements to use, the baseline measurement from Day 0 (i.e., prior to treatment) will be carried forward. If it is not possible to impute the missing values (as there is no baseline result), the standardized endogenous creatinine clearance values will be averaged based on the days available (i.e. if baseline, Day 1 and Day 2 are missing, the sum of the values from Day 3 to Day 7 will be divided by 5 – as there is 5 available days with non-missing data after imputation.

8.1.1. Primary Analysis

The primary efficacy analysis will be an analysis of variance (ANOVA) of the area under the time-corrected endogenous creatinine clearance curve from Day 1 to Day 7 with treatment and site as explanatory variables.

For all patients recruited up to the interim analysis, the primary efficacy analysis will be conducted for the ITT Combined Set at the interim analysis (which is identical to the ITT Part 1 Interim set at the time of the interim analysis) where $AUC_{1\text{-}7}$ will be compared between the 3 active treatment groups and placebo. This analysis will be considered in conjunction with the safety data to determine the optimal recAP dose for use in Part 2.

The primary efficacy analysis will be conducted for the ITT Part 2 Set, PP Day 1 – 7 Part 1 and PP Day 1 – 7 Part 2 Set where $AUC_{1\text{-}7}$ will be compared between the optimal recAP dose and placebo (for the PP Day 1 -7 Combined Set, each dose of recAP will be compared to Placebo). Also, all doses combined will be compared to Placebo.

Confirmatory testing of the single hypothesis comparing optimal dose with placebo will be based on a closed-testing procedure. This hypothesis will be rejected at the 5% significant level if it and all intersection hypotheses involving it are all rejected at the 5% significance level. The testing strategy used to combine results from Parts 1 and 2 will be a combination test based on the inverse normal method, with the test statistic of the combination test calculated as (Bauer and Köhne 1994):

$$\sqrt{\frac{n_1}{n}}\,\Phi^{-1}(1-p_1) + \sqrt{\frac{n_2}{n}}\,\Phi^{-1}(1-p_2)$$

where n_1 and n_2 are the sample sizes per group in interim analysis at Part 1 and in Part 2, respectively, $n = n_1 + n_2$, Φ refers to the standard normal distribution, and p_1 and p_2 are the one-sided p-values from interim analysis at Part 1 and from Part 2, respectively. For the intersection hypotheses, p_1 is the Dunnett adjusted one-sided p-value in Part 1 (for the optimal dose selected), and p_2 is the one-sided unadjusted p-value to compare optimal dose with placebo in Part 2 for all hypotheses.

Results from the primary analysis for interim analysis at Part 1 and for Part 2 will include least square means for each treatment and the difference in least squares means between the treatment groups will also be presented along with the associated 95% CI. P-values will be presented for least square mean treatment differences. The one-sided p-value for the combined analysis will be presented with the sample sizes and one-sided p-values from the interim analysis at Part 1 and at Part 2.

Descriptive statistics for the primary endpoint and mean standardized endogenous creatinine clearance at Day 1 to Day 7 will also be presented in a summary table by

treatment. This will be presented for all four treatment groups for the ITT Combined, ITT part 1 Interim, PP Day 1-7 Part 1, and also for the optimal recAP dose and placebo treatment groups for the ITT Part 2 and PP Day 1-7 Part 2 Sets. Analysis on the PP Set will be used as a supplement to the ITT analysis. The ITT Part 1 Interim Set (for all four treatment groups) will also be produced to help check the assumption of the combined analysis that patients recruited in each part of the study belong to the same overall patient population.

The primary endpoint, AUC_{1-7}, and standardized endogenous creatinine clearance at Day 1 to Day 7 will be listed.

A time-course plot of standardized endogenous creatinine clearance with associated error bars on each day will be presented for the optimal recAP dose and placebo groups. This plot will be repeated three times using different selection criteria for the data based on data imputation and adjudication of creatinine clearance data:

1) Including only calculated values (i.e.no imputed values)
2) Including all (non-adjudicated) calculated and imputed values
3) Including only adjudicated calculated and imputed values

The combined ITT analysis primary endpoint summary and analysis will be repeated as a supportive sensitivity analysis, excluding patients who died before day 8, with SAS® output from Part 1 and Part 2 models included on the primary combined analysis output. Additionally, descriptive statistics for the primary endpoint and mean time-corrected endogenous creatinine clearance at Day 1 to Day 7 will be presented in a summary table for the ITT Combined Set by treatment group and subgroup category, defined in Section 8.1.5.

8.1.2. Adjudication Committee

An adjudication committee will review the standardized endogenous creatinine clearance data on a case-by-case basis for part 1 patients in the same way as for the part 2 data review to determine the values to be used in the analysis. The adjudication committee will be provided with a data listing of collection time of urine and blood creatinine, their results as well as the calculated standardized endogenous creatinine clearance (more details are included in the adjudication committee charter). Additionally the adjudication committee will be provided with a data listing of collection time or urine urea nitrogen, volume of urine and BUN as well as the calculated value for urea clearance. The adjudication committee will also be provided with patients figures for standardized creatinine clearance and urea clearance.

The adjudication committee will then indicate for each record if the standardized endogenous creatinine clearance is acceptable (i.e. can be used in the analysis) and provide

comments (if applicable). PPD Biostatistics will use the standardized endogenous creatinine clearance deemed acceptable by the adjudication committee to re-derive the AUC1-7.

8.1.3. Assumption Testing

The underlying assumptions for ANOVA (normality and homogeneity of variance of the studentized residuals) will be investigated at the Part 1 interim analysis and at the Part 2 analysis by examining a normal probability plot of the residuals and a plot of the fitted values against the residuals. These results will be presented as a SAS® output.

Should there be a strong indication that these assumptions are not satisfied, a corresponding non-parametric analysis will be conducted to calculate the p-values for interim analysis at Part 1 and at Part 2 (i.e., using ANOVA with the rank-transformed values of AUC_{1-7} as the outcome variable and treatment and site as explanatory variables).

8.1.4. Sensitivity Analyses

The interim analysis was conducted on non-adjudicated data, so will be repeated using only the data deemed acceptable by the adjudication committee, therefore a sensitivity analysis will be performed on the primary endpoint using adjudicated Part 1 data.

In order to investigate the effect of imputing the missing data with LOCF, a sensitivity analysis will be conducted on the primary efficacy endpoint. The ANOVA analysis will be performed as described in Section 8.1.1 but excluding any patients that died prior to Day 8 of the study. The descriptive statistics for the primary endpoint will also be repeated for this sensitivity analysis.

In addition, the ANOVA analysis (as described in Section 8.1.1) will be repeated including as additional covariates the baseline serum creatinine results and the baseline APACHE II score for the ITT Combined Set for the optimal recAP dose and Placebo. If the analysis does not converge, APACHE score will be categorized into two categories (< 25, ≥25).

Finally, the area under the time-corrected endogenous creatinine clearance curve from Day 5 to Day 7 (AUC_{5-7}) will be derived and analyzed with an ANOVA as described in Section 8.1.1 to determine if the AUC_{5-7} is a predictor of treatment effect.

AUC_{5-7} is calculated as the average of the standardized endogenous creatinine clearance values over the 3 days. Specifically, denoting C_i as the standardized endogenous creatinine clearance on Day i, AUC_{5-7} is defined as:

$$AUC_{5-7} = \frac{1}{3}\sum_{k=5}^{7} C_i$$

Any missing C_i values will be handled by linear interpolation where possible, otherwise they will be imputed by last observation carried forward (LOCF). When there are no preceding post-baseline measurements to use, the baseline measurement from Day 0 (i.e., prior to treatment) will be carried forward. If there are missing values the values imputed for deriving AUC_{1-7} will be used to derive AUC_{5-7}.

8.1.5. Supportive Analysis for the Primary Efficacy Endpoint

Due to the high variability of the creatinine clearance, the change from baseline in serum creatinine at Day 7 will be analyzed using an ANOVA with baseline serum creatinine, treatment (optimal recAP dose and placebo) and site as explanatory variables. The analysis will be run on the ITT Combined Set. If there is more than one serum creatinine result collected at Day 7, the average at Day 7 will be used to derive the change from baseline.

Additionally the primary endpoint analysis will be repeated with the following variables as additional factors within the ANOVA model for the ITT Combined Set (for the optimal recAP dose and placebo):

- Baseline biomarkers for kidney function:
  - Fractional excretion of sodium (<1%, ≥1 % - ≤2%, >2%).
  - Fractional excretion of urea (<35%, ≥35%).

- Baseline AKIN stage (stage 1, stage 2 and stage 3).

- Microbial infection. The following are the five possible outcomes:

  1. Gram positive
  2. Gram negative
  3. Mix gram positive/gram negative
  4. Other (e.g. fungal)
  5. Unknown.

  If a blood culture is available this is leading. (Note that if a staphylococcus epidermis is the outcome this is a contamination and should be ignored). If no blood culture is available, the culture from the site for which the patient was hospitalized (index indication) is leading e.g. patient hospitalized for lung infection, with positive sputum and positive urine culture: sputum is then leading.

- Baseline urinary biomarkers for proximal tubular cell damage:

- KIM-1 (<Q1, ≥Q1 - <Q2, ≥Q2 - <Q3, ≥Q3).
- IL-18 (<Q1, ≥Q1 - <Q2, ≥Q2 - <Q3, ≥Q3).
- GST-alpha (<Q1, ≥Q1 - <Q2, ≥Q2 - <Q3, ≥Q3).

For GST-alpha, two different kits were used in the testing of GST-alpha therefore quartiles will be calculated separately for each kit and then for each subject, their baseline value will be compared against the quartile for the kit that was used to assess their GST-alpha baseline result. The resulting assigned quartile categories will then be combined together for subgroup summaries regardless of which kit was used.

- Baseline biomarkers for systemic inflammation:
  - IL-6 (<Q1, ≥Q1 - <Q2, ≥Q2 - <Q3, ≥Q3).
  - CRP (<Q1, ≥Q1 - <Q2, ≥Q2 - <Q3, ≥Q3).
  - LBP (<Q1, ≥Q1 - <Q2, ≥Q2 - <Q3, ≥Q3).

- Baseline eGFR by CKD-EPI formula (<Q1, ≥Q1 - <Q2, ≥Q2 - <Q3, ≥Q3).

- Time from first diagnosis of SA-AKI to start of recAP treatment (0 to <6 hours, 6 to <12 hours, 12 to <18 hours, 18 to <24 hours, ≥24 hours).

- Baseline APACHE II score (<25, ≥25).

Summaries will be produced with these factors as by variables.

All subgroup analyses will be exploratory; meaning that no adjustment for multiplicity is required.

A table will be produced for the ITT Combined Set displaying the number and percentage of patients included in each subgroup/factor category, by treatment group (optimal recAP dose and placebo). This will be repeated for the ITT Part 1 Interim Set and the ITT Part 2 Set.

8.2. Secondary Efficacy Endpoint

8.2.1. Key Secondary Efficacy Endpoint

The key secondary endpoint is RRT incidence during the period Day 1 (after first treatment) to Day 28, inclusive. Should a patient die or withdraw from the study during this period without recording RRT incidence, he or she will be counted as having not required RRT.

Analysis of this key secondary endpoint will be performed for the ITT Combined Set and PP Day 1– 7 Combined Set.

The optimal recAP dose will be formally compared with placebo using a logistic regression model with treatment group and site as explanatory variables, if a statistically significant result is obtained from the combination test analysis of the primary endpoint. Otherwise the results will be reported as exploratory analyses only. In the event that there are issues with the model (e.g. quasi-complete separation of data points) the reason will be investigated and if needed, site will be removed from the model. In this case, it will be documented as a deviation from the protocol in order to avoid a questionable validity of the model fit.
This endpoint will additionally be summarized for all four treatment groups using counts and percentages. The odds ratio and associated 95% confidence interval from the comparisons of the optimal recAP dose versus Placebo will also be presented. The counts and percentages will also be presented for ITT Part 1 Interim Set and ITT Part 2 Set separately.

The analysis of this key secondary endpoint will be repeated excluding patients who died or withdrew from the study prior to completion of this 28 Day period. If a statistically significant result is obtained from the combination test analysis of the primary endpoint and the key secondary endpoint, the optimal recAP dose will be formally compared with placebo using a logistic regression model with treatment group and site as explanatory variables.

The analysis of RRT incidence for the ITT Combined Set will also be presented for the supportive factor analysis as described in section 8.1.5 for the primary endpoint.

RRT data will be presented in a listing.

8.2.2. Other Secondary Efficacy Endpoints

Unless stated otherwise all analysis on the other secondary endpoints will compare the optimal recAP with placebo and be based on the ITT Combined Set, i.e. include all patients randomized to those treatment groups during Part 1 and Part 2 of the study. All summary statistics, counts and percentages, and data listings will be based on the ITT Combined Set, displaying results for all four treatment groups and including all patients randomized during Part 1 or Part 2 of the study.

8.2.2.1. Renal

Volume of Urine, Serum Creatinine, BUN/Urea Clearance, Proteinuria

A summary table will present descriptive statistics for the following renal endpoints: volume of urine (daily from Day 1 to Day 7, inclusive, and on Day 14, 21 and 28 if reliable 6 hour urine collection is possible); Serum creatinine, proteinuria, blood urea nitrogen (BUN) and urea clearance (daily from Day 1 to Day 7, inclusive, and on Day 14, 21 and 28 and on Day 60 and 90 for serum creatinine and proteinuria).

Urine volume will be collected over a 6 hour period +/- 1 hour period, and converted to mL/hour by PPD Biostatistics. Urine output (mL/hour) will be calculated using the following formula:

$$Urine\ output\ (mL/hour) = \frac{Urine\ output\ collected\ (mL)}{(Stop\ date/time\ of\ Urine\ Collection - Start\ date/time\ of\ Urine\ Collection, in\ hours)}$$

The calculated values per hour will be used in the tables and figures.

Urea clearance (mL/min) will be calculated by PPD Biostatistics using the following formula.

$$\frac{\text{Urine Urea Nitrogen (mg/dL)}}{\text{BUN (mg/dL)}} \times \frac{\text{Total urine volume collected (mL)}}{\text{Stop date/time – Start date/time of urine collection (min)}}$$

- The urine urea nitrogen (mg/dL) and BUN (mg/dL) assessed by the central laboratory will be used to determine the urea clearance.
- Per protocol, two blood samples are collected to evaluate the BUN, one at the beginning of the urine sample collection and the other at the end of the urine sample collection. The average of these two BUN measurements will be used in the formula above to assess the urea clearance. In case there is only one BUN sample available, then the BUN concentration from that one sample will be used in the formula.
- The number of minutes from the start date/time to the stop date/time of urine sample collection and the total urine volume (mL) will be derived from the Urine Volume eCRF page. If the urine volume is recorded as 0 mL, then the urea clearance will be 0 mL/min and will not be set to missing.

A time-course plot of serum creatinine, urine output and BUN/urea clearance by day will be presented separately.

All data for these endpoints will also be listed.

A summary table will present the descriptive statistics for the peak value of serum creatinine and the peak value of blood urea nitrogen (during the period Day 1 to Day 7, inclusive).

RRT

A summary table for RRT during the period Day 1 to Day 28 will be presented including the following endpoints: RRT-free days, total number of days on RRT, reasons for initiation of RRT, and reasons for stopping RRT.

An RRT-free day is a day on which a patient did not receive any form of RRT. Conversely, a day on RRT is defined as a day on which a patient received any form of RRT for any period of time on that day. For intermittent RRT, the following additional rules apply:

- Following conclusion of intermittent RRT, if the patient then has a period of six or more days before next initiation of any form of RRT, all (six or more) days in this intervening period are counted as RRT-free.
- Following conclusion of intermittent RRT, if the patient then has a period of five or less days before next initiation of any form of RRT, all (five or less) days in this intervening period are counted as RRT.

Table 8-1 Example of Derivation of RRT-Free Days and Days on RTT

On intermittent RRT	Off RRT	On RRT	RRT-free days	Days on RRT
Day 2 to Day 4 inclusive	Day 5 to Day 9 inclusive	Day 10 to Day 15 inclusive	0	14
Day 2 to Day 4 inclusive	Day 5 to Day 10 inclusive	Day 11 to Day 15 inclusive	6	8
Note: all instances of Day refer to study day				

RRT-free days from Day 1 to Day 28 will be summarized by descriptive statistics. The total number of days on RRT from Day 1 to Day 28 will also be summarized by descriptive statistics.

Reasons for initiation of RRT and reasons for stopping RRT will be summarized by counts and percentages. A patient can have more than one reason for initiation, but will only be counted once under each reason in the table if the patient reported that reason multiple times. The same approach will be applied for patients who report more than one reason for stopping.

Exploratory analysis of the total number of days on RRT will be undertaken for the comparison of the optimal recAP dose and placebo using an ANOVA with treatment and site as explanatory variables. The least square means for each treatment and the difference in least squares means between the treatment groups will also be presented along with the associated 95% CI and p-value. If a subject withdrew from study or died while still on RRT treatment before Day 28, then duration of RRT (ie, number of days on RRT) will be calculated as from RRT start date up to Day 28 (ie calculating with the "remaining days up to Day 28). For the calculation of total number of days on RRT, any missing RRT stop days will be set to either the day before the next RRT start date if more than one RRT record or to Day 28 if the patient has no further RRT records.

All RRT data will be listed.

Kidney function

The reference eGFR will be estimated by the CKD-EPI formula based on the reference serum creatinine value recorded on the AKI Diagnosis page of the eCRF for each patient. The reference serum creatinine value is recorded on the eCRF using the following order of preference:

1. Lowest value within 3 months of the hospital admission; if not available then:
2. Value at hospital admission; if not available then:
3. Value ICU/Intermediate care admission; if not available then:
4. Lowest value between 3 and 12 months prior to hospital admission

Baseline serum creatinine from the laboratory data will not be included in the calculation of reference eGFR.

Kidney function will be assessed at Baseline, Day 14, 21 and 28 by measured creatinine clearance. Baseline kidney function will only be measured by standardized creatinine clearance, however for Day 14, 21 and 28 summaries if creatinine clearance is not available then kidney function as assessed by eGFR (estimated by the CKD-EPI formula based on serum creatinine) will be used.. Kidney function will also be assessed at Day 60 and Day 90 by eGFR. Sustained loss of Kidney function, defined by eGFR < 60 mL/min, will be assessed at Day 60 and Day 90. The analyses of kidney function and sustained loss of kidney function at each timepoint will exclude any patients who die prior to the timepoint. Any other missing assessments (including early withdrawals) at Day 60 or Day 90 will be imputed by LOCF where possible, missing Day 14, 21 and 28 values will not be imputed and will be treated as missing. A summary table will present descriptive statistics for the reference kidney function and the kidney function at Baseline and at Days 14, 21, 28, 60 and 90 along with counts and percentages of patients with loss of sustained kidney function at Day 60 and Day 90. Descriptive statistics will also be presented for the change from baseline creatinine clearance value at Day 14, Day 21, Day 28, Day 60, and Day 90.

The absolute difference between placebo and each of the treatment groups will also be presented at each time point. Summaries for Day 60 and 90 will be repeated with and without the LOCF imputation of missing values applied.

All kidney function data will be listed.

Dialysis Dependency

Incidence of dialysis dependency will be assessed at Day 60 and Day 90. Patients who die or withdraw from the study prior to Day 60 or Day 90, respectively, without recording incidence of chronic dialysis will be counted as not being dialysis dependent. A summary table will present the counts and percentages of patients who are dialysis dependent at Day 60 and Day 90. Percentages for each timepoint are calculated based on the number of patients that were assessed including those who died or withdrew prior to timepoint.

All data on dialysis dependency will be listed.

8.2.2.2. Other Organs

Liver Enzymes

Liver enzymes is assessed by AST (U/L), ALT (U/L), GGT (U/L), LDH (U/L) and bilirubin (µmol/L) at Baseline, Day 1, Day 3, Day 5, Day 7, Day 14, Day 21 and Day 28.

The observed values and change from baseline for liver enzymes will be calculated by visit and summarized by descriptive statistics.

Time-course plots similar to the plot being produced for the primary endpoint will be presented for mean AST, ALT and LDH displaying the optimal recAP and placebo groups only.

All liver enzymes data will be listed.

Lung Function

Lung function is assessed, for patients on mechanical ventilation, by PaO2, P/F ratio, PEEP (cmH_2O) and tidal volume (mL/kg) daily from Day 1 to Day 28. The observed values and change from baseline for PaO2, P/F ratio, PEEP and tidal volume will be calculated by visit and summarized with descriptive statistics. For non-mechanical ventilation liters of administered oxygen will be assessed.

Table 8-2 Normal Ranges for Lung Function Parameters

Parameter	Normal Range
PCO2	4.7 – 6.4 kPa or 35-48 mmHg
PO2	10.0 – 13.3 kPa or 75 – 100 mmHg
Oxygen saturation	95 – 100%

The following conversion rate will be performed for PO2 and PCO2 where needed

kilopascal (kPa) x 7.5 = mmHg

All lung function data will be listed.

Mechanical Ventilation from Day 1 to Day 28

A ventilator-free day is defined as a day on which a patient was not on ventilator (invasive or non-invasive mechanical ventilation). The start and stop dates of the patient been under mechanical ventilation is collected in the eCRF, therefore days not on ventilator correspond to days that are outside of the start and stop dates of when the patient is on mechanical ventilation. If the method of mechanical ventilation is changed with no interruption, it corresponds to the same episode of mechanical ventilation.

Should a patient die or withdraw prior to Day 28, the days remaining in this period will be counted as per the status of the patient at time of death or withdrawal.

Ventilator-free days are calculated as 28 days minus the number of days a patient is on ventilator during this period. Descriptive statistics will be presented for the number of ventilator-free days.

Time to being off ventilator from Day 1 to Day 28, for those patients that are on ventilator at Day 1, will be compared between treatment groups by displaying Kaplan-Meier plots. Time to being off ventilator is calculated as:

Date of being off ventilator – Start date of study drug infusion + 1.

For patients that have been off ventilator and had to be put back on ventilator the latest case of ventilation will be used to calculate time to being off ventilator. Patients that do not have an off ventilator date will be censored at the earliest of Day 28 or date of study withdrawal. Patients not on ventilator at the start of study drug infusion will be excluded from this analysis.

A table of the corresponding Kaplan-Meier estimates will be presented displaying the number of patients at risk, number of patients censored, number of patients off-ventilator,

cumulative number of patients off-ventilator, Kaplan-Meier estimate, and standard errors of the Kaplan-Meier estimates. Summary statistics (based on the Kaplan-Meier analysis) for the time to being off ventilator will also be included in a separate table.

Exploratory analysis of time to being off-ventilator will be undertaken for the comparison of the optimal recAP dose and placebo using a log rank test stratified by site. The hazard ratio, associated 95% confidence interval and p-value from the comparisons of the optimal recAP dose versus Placebo will be presented.

The number and percentage of patients not on mechanical ventilation at baseline, requiring and not requiring mechanical ventilation during the first 28 days will also be presented.

Mechanical and non-mechanical ventilation data will be listed.

Shock-free from Day 1 to Day 28

Shock-free is defined as a patient that is not on vasopressors or inotropic agents. Shock-free days and Time to being shock-free will be analyzed in an identical fashion to the method described above.

Shock-free data will be listed.

SOFA

The SOFA questionnaire assesses 6 systems organs: respiratory system, central nervous system, cardiovascular hypotension, liver, coagulation and renal system. Each system has 5 response options ranging from 0 to 4 that reflect increasing levels of failure. Any response > 0 is classed as a dysfunctional organ.
The SOFA score is assessed at baseline, and at all planned visits from Day 1 to Day 28 (as long as the patient is in the ICU/Intermediate care) and at discharge from ICU/Intermediate care. The observed values and change from baseline for total SOFA score and respiratory system, central nervous system, cardiovascular hypotension, liver, coagulation and renal system individually will be calculated by visit and summarized with descriptive statistics.

A time-course plot similar to the plot being produced for the primary endpoint will be presented for the mean SOFA score displaying the optimal recAP and placebo groups only.

The number of dysfunctional organs for each patient will be summarized with counts and percentages by visit.

All SOFA data will be listed.

Deaths during the 90 day study period

Mortality at Day 28 and Day 90 will be summarized by the counts and percentages of patients who died prior to or at these timepoints. Exploratory analysis will also be undertaken to compare the number of deaths between the optimal recAP dose and placebo at Day 28 and Day 90 using a Cochran-Mantel-Haenzel (CMH) test. The CMH test statistic and the corresponding p-value will be presented.

Should either of the CMH tests at Day 28 and Day 90 produce a significant result, time to death from Day 1 to Day 90 will be compared between treatment groups by displaying Kaplan-Meier plots. Time to death is calculated as:

Date of death – Start date of study drug infusion + 1.

Patients that do not die will be censored at the earliest of Day 90 or date of study withdrawal. A table of the corresponding Kaplan-Meier estimates will be presented displaying the number of patients at risk, number of patients censored, number of deaths, cumulative number of deaths, Kaplan-Meier estimate, and standard errors of the Kaplan-Meier estimates. Summary statistics (based on the Kaplan-Meier analysis) for the time to death will also be included in a separate table.

Purine Data

Purine data will be listed along with urine volume and urine sample collection start date and collection times for the Iohexol set.

8.2.2.3. Biomarkers

Kidney Function Biomarkers

Kidney function biomarkers are assessed by: sodium and fractional excretion of sodium at Baseline and Day 1 to Day 7; urine creatinine, urinary BUN/urea, urea clearance, fractional excretion of urea and urine output at Baseline and Day 1 to Day 28; and serum creatinine, BUN, and proteinuria at Baseline and Day 1 to Day 90.

The observed values and change from baseline for kidney function markers will be calculated by visit and summarized with descriptive statistics.

All kidney function markers will be listed.

Urinary Biomarkers for Proximal Tubular Cell Damage

Proximal tubular damage is assessed by the following urinary biomarkers: KIM-1, IL-18 and GST-alpha (urinary concentration and normalized for urinary creatinine concentration) at Baseline and each scheduled visit up to Day 28.
The observed values and change from baseline for urinary biomarkers will be calculated by visit and summarized with descriptive statistics.

Time-course plots similar to the plot being produced for the primary endpoint will be presented for mean KIM-1, IL-18 and GST-alpha, displaying the optimal recAP and placebo groups only.

As described in Section 8.1.5, two different kits were used for GST-alpha testing and therefore data will be summarized separately depending on which kit was used to test.

All urinary biomarkers will be listed.

Biomarkers for Systemic Inflammation

Biomarkers for systemic inflammation are assessed by IL-6, CRP and LBP at Baseline and each scheduled visit up to Day 28.

The observed values and change from baseline for biomarkers for systemic inflammation will be calculated by visit and summarized with descriptive statistics.

Time-course plots similar to the plot being produced for the primary endpoint will be presented for mean IL-6, CRP and LBP, displaying the optimal recAP and placebo groups only.

All biomarkers for systemic inflammation will be listed.

8.3. Other Efficacy Endpoints

Composite Endpoints

Patients that meet, or do not meet at least 1 of the following criteria will be summarized by counts and percentages for each composite endpoint:

Composite Endpoint 1:

1. Received RRT (prior to Day 28 [inclusive]) or
2. Died (prior to Day 28 [inclusive])

Composite Endpoint 2:

1. eGFR < 60 mL/min (at Day 60, estimated by the CKD-EPI formula based on serum creatinine) or
2. Dialysis dependency (up to Day 60) or
3. Died (prior to Day 60)

Composite Endpoint 3:

1. eGFR < 60 mL/min (at Day 90, estimated by the CKD-EPI formula based on serum creatinine) or
2. Dialysis dependency (up to Day 90) or
3. Hospitalized for a new episode of AKI (prior to Day 90) or
4. Died (prior to Day 90)

Exploratory analysis of each composite endpoint will be undertaken for the comparison of the optimal recAP dose and placebo using a logistic regression model with treatment group and site as explanatory variables. The odds ratio and associated 95% confidence interval from the comparisons of the optimal recAP dose versus Placebo will be presented together with the p-value for this comparison.

Serology

Serology is assessed by IgG, IgE, and total immunoglobulin at Baseline, Day 14 and Day 28.

The observed values and change from baseline for IgG, IgE, and total immunoglobulin will be calculated by visit and summarized with descriptive statistics.

All Serology data will be listed.

Anti-Drug Antibody Data

All anti-drug antibody data will be listed.

Quality of Life

Quality of Life is assessed by the EQ-5D questionnaire at Baseline, ICU/Intermediate care discharge, and Day 90.

The questionnaire assesses 5 dimensions: mobility, self-care, usual activities, pain/discomfort and anxiety/depression. Each dimension has 5 response options (no problems, slight problems, moderate problems, severe problems, and extreme problems) that reflect increasing levels of difficulty. The patient will be asked to indicate his/her current health state by selecting the most appropriate level in each of the 5 dimensions.

The questionnaire also includes a visual analogue scale, where the patient will be asked to rate current health status on a scale of 0-100, with 0 being the worst imaginable health state.

The EQ-5D responses from each dimension will be summarized with counts and percentages by treatment group and visit.
The observed values and change from baseline in visual analog scale will be calculated by visit and summarized with descriptive statistics.

All EQ-5D data will be listed.

Discharge from ICU/Intermediate care for Day 1 to Day 28 and Day 1 to Day 90

Total time in ICU/Intermediate care is calculated as the number of days a patient has been in the ICU/Intermediate care from the start of first study drug infusion to discharge where discharge is defined as the time of actual transfer (up to Day 28 and Day 90) and will be summarized by the descriptive statistics. For the calculation of Total time in ICU/Immediate care for Day 1 to 28 if a subject discontinued or died prior to Day 28 whilst still in ICU, the ICU discharge date is assumed as Day 28. For the calculation of Total time in ICU/Immediate care for Day 1 to 90, if a subject discontinued or died whilst still in ICU, then the discharge date will be assumed as taking the maximum of Day 28 or study discontinuation date.

Time to being discharged from ICU/Intermediate care will be compared between treatment groups by displaying Kaplan-Meier plots, where discharge is defined as the time when the decision was made to transfer the patient (as opposed to the time of actual transfer). Time to being discharged from ICU/Intermediate care is calculated as:

Planned discharge date from ICU/Intermediate care – Start date of study drug infusion + 1.

For patients that have been discharged from the ICU/Intermediate care and had to be readmitted, only their first case of admission will be used to calculate time to being discharged from the ICU/Intermediate care. Patients that have transferred to another ICU/Intermediate care will not be classed as discharged from the ICU/Intermediate care. Patients that have not been discharged from the ICU/Intermediate care will be censored at Day 28 (when died or discontinued prior to Day 28) or on the date of study withdrawal (when died or discontinued after Day 28).

A table of the corresponding Kaplan-Meier estimates will be presented displaying the number of patients at risk, number of patients censored, number of patients discharged from ICU/Intermediate care, cumulative number of patients discharged from ICU/Intermediate care, Kaplan-Meier estimate, and standard errors of the Kaplan-Meier

estimates. Summary statistics (based on the Kaplan-Meier analysis) for the time to discharge from ICU/Intermediate care will also be included in a separate table.

Exploratory analysis of time to being discharged from the ICU/Intermediate care will be undertaken for the comparison of the optimal recAP dose and placebo using a log rank test stratified by site. The hazard ratio, associated 95% confidence interval and p-value from the comparisons of the optimal recAP dose versus Placebo will be presented.

Discharge from ICU/Intermediate care data will be listed.

Discharge from Hospital for Day 1 to Day 28 and Day 1 to Day 90

Total time in hospital and Time to being discharged from hospital will be analyzed in an identical fashion to the method described above, except the stratified log-rank test will not be performed. Patients that have transferred to another hospital will not be classed as discharged from the hospital. For the calculation of Total time in hospital for Day 1 to 28, if a subject discontinued or died prior to Day 28 whilst still in hospital, the hospital discharge date is assumed as Day 28. For the calculation of Total time in hospital for Day 1 to 90, if a subject discontinued or died whilst still in hospital, then the discharge date will be assumed as taking the maximum of Day 28 or study discontinuation date.

Iohexol substudy

Para-aminohippuric (PAH) will not be included in any analyses or data listings because this product is no longer manufactured.

A single intravenous bolus injection of 5 mL iohexol (Omnipaque 240 mg/mL) will be administered over 5 minutes on Day 1 and on Day 7 or discharge from the ICU, whichever comes first. The bolus will be administered at the start of the 6 ± 1 hour urine collection interval. For the measurement of iohexol, 2 mL blood samples will be collected in EDTA anticoagulated Vacutainer® tubes at the following time points: prior to iohexol bolus administration and at 60, 120, and 360 minutes.

$CL_{Iohexol}$ will be calculated using the concentration values from all time points according to a two-compartment or one-compartment model using the formula:

$CL_{Iohexol}$= Dose/AUC (with AUC: area under the plasma concentration–time curve).

Then, in the case of a one compartment model, plasma clearance of iohexol will be modified for the early distribution phase by the BrÖchner–Mortensen (BM) formula:

$CL_{Iohexol}$ BM = (0.990778 ×$CL_{Iohexol}$) – (0.001218 × $CL_{Iohexol}^2$) (Gaspari et al, 1995).

Iohexol plasma concentrations will be listed and summarized using descriptive statistics by timepoints. Iohexol PK parameters will be listed and summarized using descriptive statistics. These summaries will include the number of patients (n), mean, standard deviation (SD), coefficient of variation (CV), minimum, median, maximum, geometric mean, and geometric %CV, when applicable.

Iohexol clearance ($CL_{Iohexol}$), as a gold standard measure of GFR, will be correlated to creatinine clearance in a subset of patients using scatter plots.

Exogenous Creatinine Clearance

To evaluate if renal replacement therapy (RRT) has an effect on endogenous creatinine clearance, the exogenous creatinine clearance by RRT equipment will be assessed by measuring the creatinine concentration in the effluent and effluent volume during the 6 ± 1 hour urine collection interval. This will be only done by the sites participating in the Iohexol substudy.

Standardized exogenous creatinine clearance (mL/min) by the RRT equipment will be calculated by PPD Biostatistics using the following formula.

$$\frac{\text{Effluent Creatinine (mg/dL)}}{\text{Serum Creatinine (mg/dL)}} \times \frac{\text{Total effluent volume collected (mL)}}{\text{Stop date/time – Start date/time of effluent collection (min)}}$$

- The effluent creatinine (mg/dL) will be assessed by the laboratory conducting the Ioxehol data.
- The serum creatinine (mg/dL) assessed by the central laboratory and used in the primary endpoint of endogenous creatinine clearance will be used to determine the standardized exogenous creatinine clearance.
- Per protocol, two blood samples are collected to evaluate the serum creatinine, one at the beginning of the urine sample collection and the other at the end of the urine sample collection. The average of these two serum creatinine measurements will be used in the formula above to assess the standardized exogenous creatinine clearance. In case there is only one serum creatinine sample available, then the serum creatinine concentration from that one sample will be used in the formula.
- The number of minutes from the start date/time to the stop date/time of effluent sample collection and the total effluent volume (mL) from the RRT equipment will be derived from data collected at site.

The time-corrected exogenous creatinine clearance will be listed for subjects who are on RRT in the Iohexol population.

A time-course plot of exogenous creatinine clearance on each day will be presented for each subject on RRT in the Iohexol population.

9. Safety Analysis

Safety will be assessed by evaluation of the following variables:

- Adverse events (AE)
- Local Laboratory (Hematology, Clinical Chemistry and Urinalysis parameters not considered in the efficacy analyses)
- Vital Signs
- Physical Examination findings including ECGs

All analyses of safety will be conducted using the Safety Set and will be presented for all 4 treatment groups, for both parts of the study.

9.1. Adverse Events

An AE is defined as any untoward medical occurrence in a patient enrolled into a clinical study regardless of its causal relationship to study drug.

A Treatment emergent Adverse Event (TEAE) is defined as any event not present before exposure to study drug or any event already present that worsens in either intensity or frequency after exposure to study drug.

All AEs will be coded using MedDRA Version 16.1 or later.

9.1.1. Incidence of Adverse Events

Summaries of the total number of TEAEs and the number and percentage of patients with at least one TEAE will be provided by treatment group. Treatment emergent AEs will be presented by SOC and PT. At each level of patient summarization, a patient is counted once if the patient reported one or more events.

All AEs will be presented in a listing.

9.1.2. Relationship of Adverse Events to Study Drug

The investigator will provide an assessment of the relationship of the event to the study drug. The relationship of the event to the study drug will be characterized using the following classification and criteria:

Unrelated: This relationship suggests that there is no association between the study drug and the reported event.

Possible: This relationship suggests that treatment with the study drug caused or contributed to the AE; i.e., the event follows a reasonable temporal sequence from the time of drug administration or follows a known response pattern to the study drug, but could also have been produced by other factors.

Probable: This relationship suggests that a reasonable temporal sequence of the event with drug administration exists and, based upon the known pharmacological action of the drug, known or previously reported adverse reactions to the drug or class of drugs, or judgment based on the investigator's clinical experience, the association of the event with the study drug seems likely. The event disappears or decreases on cessation or reduction of the dose of study drug.

Definite: This relationship suggests that a definite causal relationship exists between drug administration and the AE, and other conditions (concurrent illness, progression/expression of disease state, or concurrent medication reaction) do not appear to explain the event. The event reappears or worsens if the study drug is re-administered.

TEAEs that are missing a relationship will be presented in the tables as "Definite".

The TEAE data will be categorized and presented by SOC, PT, and relationship in a manner similar to that described in Section 9.1.1. In the TEAE relationship table, at each level of patient summarization, a patient is counted once for the most related event if the patient reported one or more events.

9.1.3. Severity of Adverse Event

The intensity of an AE refers to the extent to which an AE affects the patient's daily activities and will be rated as mild, moderate, or severe using the following criteria:

Mild: These events require minimal or no treatment and do not interfere with the patient's daily activities.

Moderate: These events are sufficiently discomforting to interfere with normal activities.

Severe: These events interrupt a patient's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually incapacitating.

TEAEs that are missing severity will be presented in tables as "Severe" but will be presented in the data listing with a missing severity.

The TEAE data will be categorized and presented by SOC, PT, and severity in a manner similar to that described in Section 9.1.1. In the table, at each level of patient summarization, a patient is counted once for the most severe event if the patient reported one or more events.

9.1.4. Serious Adverse Events

The seriousness of an AE should be assessed by the Investigator independently from the severity of the AE. A SAE is defined as any untoward medical occurrence that at any dose results in death, is life-threatening, is a congenital anomaly/birth defect, requires in-patient hospitalization or prolongation, or results in significant disability/incapacity.

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered SAEs when, based upon medical judgment, they may jeopardize the patient and may require medical or surgical intervention to prevent one of the outcomes listed above.

Serious treatment emergent adverse events (TEAE) will be presented in a table. They will be presented by SOC and PT in a manner similar to that described in Section 9.1.1.

9.1.5. Adverse Events Leading to Treatment Discontinuation

A summary of TEAEs with a study drug action taken of “Treatment Withdrawn” will be presented in a table. They will be presented by SOC and PT in a manner similar to that described in Section 9.1.1.

9.1.6. Death

For patients who died any time during the study or during follow-up, their cause of death will be categorized and presented by SOC, PT. A listing of all deaths and associated cause of death will be provided as well. All death will be coded using MedDRA Version 16.1 or later.

9.2. Laboratory Evaluations

Laboratory assessments not used for the efficacy analysis will be used for the safety analysis and be performed by the local laboratory. All summaries will be based on the units provided defined in the eCRF, no conversion will be done. Also, the out of normal range data will be presented.

Hematology and clinical chemistry assessments will be summarized according to the visits and will be assessed at Baseline, Day 1, 3, 5, 7, 14, 21 and 28. Shift tables presenting baseline and post-baseline values below, within, above or below the normal range (normal ranges as provided by the local laboratories), and count tables at each visit will be presented for hematology and clinical chemistry tests with numeric values below, within or above the normal range (normal ranges as provided by the local laboratories) by treatment group for patients in the Safety Set. The percentage will be based on the number of patients who have attended the visit.

Only scheduled assessments will be included in the laboratory summaries. In case of repeat values the last assessment will be used for pre-dose visits and the first value for post-dose visits.

9.2.1. Hematology

The following laboratory tests will be included: hemoglobin, hematocrit, leukocytes, differential leukocytes (absolute values and as a percentage of total leukocytes), erythrocytes, thrombocytes and activated partial thromboplastin time**.** All hematology data by patient will be presented in a listing.

9.2.2. Clinical Chemistry

The following laboratory tests will be included**:** arterial pH, CRP, urea, creatinine, creatine phosphokinase, total protein, albumin, glucose, sodium, potassium, chloride, bicarbonate, and lactate**.** All chemistry data by patient will be presented in a listing.

The following normal range will be used for arterial pH: 7.35 – 7.45

9.2.3. Urinalysis

No urinalysis will be performed as part of the safety analysis, all urinalysis parameters will be analyzed as part of the efficacy analysis (see Section 8.2.2.3).

9.2.4. Pregnancy

Either a blood human chorionic gonadotropin (hCG) or urine hCG (dipstick) pregnancy test will be performed for all females of childbearing potential at baseline. All pregnancy data by patient will be presented in a listing.

9.3. Vital Sign Measurements

Vital signs are assessed at Baseline, Day 1, 2, 3, 4, 5, 6, 7, 14, 21 and 28, where systolic blood pressure (mmHg), diastolic blood pressure (mmHg), temperature (°C), heart rate (beats/minute), oxygen saturation (%) and respiratory rate (breaths/minute) are recorded.

For Day 1, 2 and 3, vital signs excluding temperature are assessed immediately prior to the start of infusion of study drug, 5 minutes after start of infusion, 30 minutes after start of infusion, immediately after the completion of the study drug infusion, 30 and 60 minutes after completion of infusion. On day 1 only, vital signs will additionally be assessed 2 hours, 3 hours, 4 hours and 5 hours after completion of study drug infusion.

Summary tables presenting observed values and changes from pre-dose will be presented for vital sign data at pre-dose, 5 minutes after start of infusion, 30 minutes after start of infusion, immediately post-dose and 30 and 60 minutes post-dose, 2 hours post-dose, 3 hours post-dose, 4 hours post-dose, 5 hours post-dose for Day 1, at pre-dose, 5 minutes after start of infusion, 30 minutes after start of infusion, and 30 and 60 minutes post-dose for Day 2 and 3 by treatment group for patients in the Safety Set.

Summary tables presenting observed values and changes from baseline will be presented for vital sign data at Baseline, Day 1, 2, 3, 4, 5, 6, 7, 14, 21 and 28 by treatment group for patients in the Safety Set. Additionally, counts and percentages of patients with temperature <36^{o}C, >=36 ^{o}C to <=38^{o}C and >38^{o}C will be presented by treatment group for each timepoint at which temperature is assessed.

A time-course plot of vital signs at the interim analysis with associated standard error bars for each timepoint will be presented for all three recAP doses and placebo groups.

All vital sign data by patient will be presented in a listing.

9.4. Physical Examination

Physical examination is assessed at baseline and at all subsequent visits up to the Day 28 visit. Each visit captures the status of a body system and any finding associated with the body system as normal, abnormal, or not done. The following body systems are captured: heart, lungs, head, neck, abdominal, neurological-non-MS (i.e., neurological findings not related to multiple sclerosis) skin, extremities and others (if applicable). Physical examination results for all patients will be presented in a listing.

9.5. Electrocardiogram

All patients will have a 12-lead ECG performed at baseline, Day 3, and Day 14.

Summary tables presenting observed values and changes from baseline will be presented for ventricular rate (bpm), PR Interval (msec), QRS Duration (msec), QT Interval (msec), QTcB Interval (msec), by treatment group for patients in the Safety Set. Changes from baseline to each scheduled post-baseline visit will be presented.

The ECG interpretations will be summarized in shift tables comparing the ECG values of each post-baseline visit with the value at the baseline visit for the Safety Set. The percentage will be based on the number of patients who have attended the visit. In addition the worst post-baseline ECG value will be compared with the value at the baseline visit (with worst of all being Abnormal and best being Normal).

Electrocardiogram data for all patients will be presented in a listing.

10. Pharmacokinetics and Pharmacodynamics

The pharmacokinetics and pharamcodynamics analyses will be described in the Population Pharmacokinetics/ Pharamcodynamics Report Analysis Plan.

11. Interim Analysis

An unblinded interim analysis will be conducted on the Part 1 data to determine the optimal recAP dose for Part 2. This analysis will compare the 4 treatment groups from Part 1 on the primary efficacy endpoint, and a selection of the safety data. The interim analysis will be conducted when the first 7 days of laboratory data have been collected for 120 patients from Part 1, unless the patient was randomized but died or discontinued prior to completing 7 days.

To maintain the blind, the interim analysis will be conducted and delivered by an unblinded PPD Biostatistics team located at a different site to the blinded PPD biostatistics personnel involved in the study. The results will be reviewed by an independent DMC, who will make the dose selection decision. A futility analysis will also be conducted at the interim analysis. If none of the 3 recAP doses in Part 1 show evidence of efficacy (i.e., all 3 groups have 1-sided, unadjusted p-value greater than 0.8), then the study will be terminated.

The following outputs will be produced for the Interim Analysis:

Table 14.1.1.1.1	Disposition at Interim Analysis All Enrolled Set
Table 14.1.1.6.1	Concomitant Medications at Interim Analysis Safety Set
Table 14.2.1.1.1	Summary of Time-Corrected Endogenous Creatinine Clearance at Interim Analysis ITT Combined Set
Table 14.2.1.1.2	Sensitivity Analysis: Summary of Time-Corrected Endogenous Creatinine Clearance at Interim Analysis – Excluding Patients Who Died Prior to Day 8 ITT Combined Set
Table 14.2.1.2.1	ANOVA for Area Under the Time-Corrected Endogenous Creatinine Clearance Curve from Day 1 to Day 7 (AUC 1-7) at Interim Analysis ITT Combined Set
Table 14.2.1.2.2	Sensitivity Analysis: ANOVA for Area Under the Time-Corrected Endogenous Creatinine Clearance Curve from Day 1 to Day 7 (AUC 1-7) at Interim Analysis – Excluding Patients Who Died Prior to Day 8 ITT Combined Set
Table 14.2.1.2.3	SAS Output for the Primary Efficacy Endpoint at Interim Analysis: ANOVA for Area Under the Time-Corrected Endogenous Creatinine Clearance Curve from Day 1 to Day 7 (AUC 1-7) ITT Combined Set
Table 14.2.2.13	Analysis of Incidence of RRT from Day 1 to Day 7 at Interim Analysis ITT Combined Set
Table 14.2.9.1	Emax – Model Parameters Estimates at Interim Analysis ITT Combined Set
Table 14.3.1.1.1	Treatment Emergent Adverse Events at Interim Analysis by System Organ Class and Preferred Term Safety Set
Table 14.3.1.1.2	Serious Treatment Emergent Adverse Events at Interim Analysis by System Organ Class and Preferred Term Safety Set
Table 14.3.1.3.1	Treatment Emergent Adverse Events at Interim Analysis by Relationship to Study Drug Safety Set
Table 14.3.2.1	Summary of Cause of Death at Interim Analysis by System Organ Class and Preferred Term Safety Set
Table 14.3.5.3.1	Hematology Shift Table by Treatment at Interim Analysis Safety Set
Table 14.3.5.3.2	Laboratory Results: Hematology at Interim Analysis Safety Set
Table 14.3.5.4.1	Clinical Chemistry Shift Table by Treatment at Interim Analysis Safety Set
Table 14.3.5.4.2	Laboratory Results: Clinical Chemistry at Interim Analysis Safety Set
Table 14.3.6.2.1	Change from Baseline in Vital Signs at Interim Analysis Safety Set
Table 14.3.7.1.1	APACHE II Score at Interim Analysis Safety Set
Figure 14.2.9.1	Emax – Model Fitting at Interim Analysis ITT Combined Set
Figure 14.3.6.2.1	Time-course plot of Vital Signs at Interim Analysis Safety Set
Listing 16.3.1.1.2	Deaths Safety Set

The DMC will conduct three additional reviews of the safety data by teleconference once the first 7 days of laboratory data are available for: 75 patients in Part 1; 60 patients in Part 2 (total of 180 patients); and 125 patients in Part 2 (total of 245 patients). In each case the

milestone will be patients with at least 7 days of laboratory data or who were randomized but discontinued or died prior to completing 7 days. The following outputs will be produced for these safety reviews:

Table 14.1.1.1.1	Disposition at Interim Analysis All Enrolled Set
Table 14.1.1.6.1	Concomitant Medications at Interim Analysis Safety Set
Table 14.3.1.1.1	Treatment Emergent Adverse Events at Interim Analysis by System Organ Class and Preferred Term Safety Set
Table 14.3.1.1.2*	Serious Treatment Emergent Adverse Events at Interim Analysis by System Organ Class and Preferred Term Safety Set
Table 14.3.1.3.1	Treatment Emergent Adverse Events at Interim Analysis by Relationship to Study Drug Safety Set
Table 14.3.2.1*	Summary of Cause of Death at Interim Analysis by System Organ Class and Preferred Term Safety Set
Table 14.3.5.3.1	Hematology Shift Table by Treatment at Interim Analysis Safety Set
Table 14.3.5.3.2	Laboratory Results: Hematology at Interim Analysis Safety Set
Table 14.3.5.4.1	Clinical Chemistry Shift Table by Treatment at Interim Analysis Safety Set
Table 14.3.5.4.2	Laboratory Results: Clinical Chemistry at Interim Analysis Safety Set
Table 14.3.6.2.1	Change from Baseline in Vital Signs at Interim Analysis Safety Set
Figure 14.3.6.2.1	Time-course plot of Vital Signs at Interim Analysis Safety Set
Table 14.3.7.1.1*	APACHE II Score at Interim Analysis Safety Set
Listing 16.3.1.1.2*	Deaths Safety Set

* Outputs were requested after the first DMC meeting.

References to 'Interim Analysis' will be removed from all titles and footnotes when outputs are provided at safety reviews.

Additional electronic reviews of AE listings provided by pharmacovigilance (PVG) will be conducted at planned time points and the DMC can request ad hoc reviews. See Table 15-2 for a full summary of the planned reviews.

12. Changes in the Planned Analysis

As part of the primary efficacy analysis an additional analysis set PP Day 1-7 Part 1 has been added. The protocol states that primary endpoint should be carried out separately for Part 1 and Part 2, however to do this analysis on the PP Set, the PP Day 1-7 Part 1 Set is needed to be defined to obtain the analysis for Part 1 of the study.

The following are changes from the Protocol Amendment v2.0:

Table 12-1 Summary of Changes from Planned Analysis and Reason

Change	Change
List of subgroups (Sections 2.3 and 8)	Microbial Infection added
Secondary Efficacy Endpoints (Section 8.2)	Contrary to Section 7.7.2 of the protocol, two sets of outputs have not been produced for these endpoints. Instead, the planned summaries have been combined to present all four treatments for all patients (i.e., per the safety analysis).
Other Secondary Endpoints (Sections 3.2.3 and 8.2.2.1)	Kidney function at Days 14 and 21 will also assessed and analyzed.
Other Endpoints (Sections 3.2.4)	Total time in ICU/Intermediate care and Total time in hospital also based on time to decision of transfer.
Other Endpoints (Section 3.2.4)	Total time in ICU/Intermediate care and Total time in hospital, based on time to decision of transfer deleted.

13. Change History

Version (date)	Section	Summary of change
1.4 (06DEC2016)	**2.3**	**FE Urea analysis added**
1.4 (06DEC2016)	**3.1**	**Schedule of events – treatment administration updated to be within 96 hrs from sepsis first diagnosis**
1.4 (06DEC2016)	**3.2.3**	**Added BUN/Urea clearance as other secondary endpoint**
1.4 (06DEC2016)	**3.2.3**	**Added kidney function assessment at Day 14, 21 and 28 as other secondary endpoint**
1.4 (06DEC2016)	**3.2.3**	**Added incidence of dialysis dependency at Day 60 and Day 90 as other secondary endpoint**
1.4 (06DEC2016)	**4**	**Added information on the assignment of patients to analysis sets who were recruited during the interim analysis**
1.4 (06DEC2016)	**4**	**Added information that all summaries will be provided by treatment arm**
1.4 (06DEC2016)	**4**	**Updated 'average dose received' and 'received treatment group assignment' categories**
1.4 (06DEC2016)	**4.3.2.2**	**ITT Part 1 Interim analysis set added**
1.4 (06DEC2016)	**4.3.2.2**	**PP Day 1 – 7 Part 1 analysis set added**
1.4 (06DEC2016)	**4.3.6**	**Iohexol analysis set added**
1.4 (06DEC2016)	**6.2**	**Acute physiology and chronic health evaluation (APACHE II) score added to baseline disease characteristics**

Version (date)	Section	Summary of change
1.4 (06DEC2016)	**6.2**	**Number and percentage of patients with vasopressor/inotropic therapy use added to baseline disease characteristics**
1.4 (06DEC2016)	**6.2**	**FE Urea added and creatinine clearance is using formula given in 8.1 section and CKD-EPI formula added in the appendix.**
1.4 (06DEC2016)	**8**	**Supportive analysis updated to include specific group categories as factors.**
1.4 (06DEC2016)	**8**	**Table added to provide an overall summary of the efficacy analyses**
1.4 (06DEC2016)	**8.1**	**Updated so only adjudicated Part 1 will be used in sensitivity analysis.**
1.4 (06DEC2016)	**8.1, 8.1.2**	**Adjudication committee review added for creatinine clearance data**
1.4 (06DEC2016)	**8.1, 8.1.2**	**Formula added for time-corrected endogenous creatinine clearance calculation**
1.4 (06DEC2016)	**8.1.4**	**Sensitivity analysis added to repeat primary endpoint analysis based on adjudicated Part 1 data. ANOVA analysis excluding any patients that died prior to Day 8 and descriptive statistics on the primary endpoint will be performed for the sensitivity analysis.**
1.4 (06DEC2016)	**8.2.2.1**	**Urine volume and urea clearance calculation formulas were added**
1.4 (06DEC2016)	**8.2.2.1**	**Details added on RRT-free day calculation method**
1.4 (06DEC2016)	**8.3**	**Added anti-drug antibody data as other efficacy endpoint**
1.4 (06DEC2016)	**8.3**	**Iohexol substudy details added**
1.4 (06DEC2016)	**8.1.5**	**Category for FE Urea added**
1.4 (06DEC2016)	**9.1.2**	**Separate summary of TEAEs where relationship to study drug is 'Possible', 'Probable' or 'Definite' removed**
1.4 (06DEC2016)	**9.2**	**Laboratory evaluations analyses modified to be based on shift tables as per normal ranges provided by the local laboratory**
1.4 (06DEC2016)	**11**	**Amended list of outputs provided for the interim analysis**
1.4 (06DEC2016)	**11**	**Added details of outputs provided for the DMC meetings**
1.4 (06DEC2016)	**Appendices 15.2, 15.3, 15.4**	**Added appendices of schedule of DMC reviews, weight ranges and pre-calculated corresponding volumes and CKD-EPI formula used for GFR calculation**
2.0 (13JAN2017)	**3.2**	**Total time in ICU/Intermediate care and Total time in hospital endpoint analysis based on time to decision of transfer deleted. Also both periods Day 1 to 28 and Day 1 to 90 added.**
2.0 (13JAN2017)	**4.3.3.1, 4.3.3.2, 4.3.3.2, 4.3.3.4**	**'Non-adherence to inclusion/exclusion criteria with enrollment of the patient' is used for eligibility criteria.**
2.0 (13JAN2017)	**8.1.1**	**Hyperlink for section 8.1.5 added.**
2.0 (13JAN2017)	**8.2.2.1**	**'Urine volume' replaced with 'Urine output' and the time course plot updated to be on urine output (ml/hr) and not on volume of urine**

Version (date)	Section	Summary of change
2.0 (13JAN2017)	**8.2.2.2**	**Sentence 'non mechanical analysis' replaced with 'non mechanical ventilation'.**
2.0 (13JAN2017)	**8.3**	**Day 1-28 and the day1-90 for ICU discharge and hospital discharge used (like section 3.2).**
3.0 (25SEP2017)	**General**	**'Time-corrected' only used for reference to AUC of Creatinine Clearance. 'Standardized' used elsewhere.**
3.0 (25SEP2017)	**2.3 8.1.5**	**Added ≥24 hour category for Time from first diagnosis of SA-AKI to start of treatment**
3.0 (25SEP2017)	**3.2.4**	**Kaplan-Meier will be done once for time to discharge from ICU and time to discharge from Hospital (removed separate analyses for Days 1-28 and Days 1-90)**
3.0 (25SEP2017)	**4**	**Definition of baseline updated for subjects who were randomized but not treated**
3.0 (25SEP2017)	**4**	**Added text on handling of < or > for laboratory values and added Table 4-2 Serum Creatinine and CRP laboratory conversions**
3.0 (25SEP2017)	**4.3.3.4 5.1 8.2.1**	**Removed PP Day 1-28 Set and replaced with PP Day 1-7 Combined Set where appropriate**
3.0 (25SEP2017)	**6.1**	**Race category updated from 'Not Applicable' to 'Not Collectable'.**
3.0 (25SEP2017)	**6.2**	**Local lab values for serum creatinine will be used if central lab values are not available.**
3.0 (25SEP2017)	**6.2**	**CKD-EPI formula will no longer be used if creatinine clearance values are not available.**
3.0 (25SEP2017)	**8.1**	**Added rule for standardized creatinine clearance calculation that when urine volume is <30ml/24 hour then standardized creatinine clearance will be set to 1ml/min.**
3.0 (25SEP2017)	**8.1.1**	**Clarification added that the p-values used in the equation for the combination test are 1-sided p-values.**
3.0 (25SEP2017)	**8.1.1**	**Time course plot for creatinine clearance will be repeated three times based on different selection based on imputation and adjudication of creatinine clearance data.**
3.0 (25SEP2017)	**8.1.2**	**Removed AUC from list of data reviewed by adjudication committee.**
3.0 (25SEP2017)	**8.1.5**	**All baseline variables of interest /subgroups will be used as additional covariates in the statistical models (not as by-groups). In addition, as a result, Baseline AKIN stages 2 and 3 will not be combined into an additional group.**
3.0 (25SEP2017)	**8.1.5**	**Updated categories for microbial infection and added rules for handling microbial infection data.**
3.0 (25SEP2017)	**8.1.5 8.2.2.3**	**GST-alpha measured using two separate kits. Quartiles will be calculated separately for the different kits**
3.0 (25SEP2017)	**8.1.5**	**Added clarification that subgroup summary tables will also be presented for ITT Part 1 Interim Set and ITT Part 2 Set**
3.0 (25SEP2017)	**8.2.1**	**For model convergence issues, site will be removed from the model and documented.**

Version (date)	Section	Summary of change
3.0 (25SEP2017)	**8.2.2.1**	**RRT: Removed exclusion of patients who died or withdrew from the study prior to Day 28 from the calculation of RRT-Free days. For the exploratory ANOVA, subjects who died before Day 28 whilst still on RRT will be assumed to have finished RRT on Day 28.**
3.0 (25SEP2017)	**8.2.2.1**	**Kidney Function: Reference eGFR will be using serum creatinine from eGFR only, Baseline Kidney Function will be assessed using creatinine clearance only. Days 14, 21 and 28 will be assessed using creatinine clearance if available or eGFR if not. LOCF will only be implemented for Days 60 and 90.**
3.0 (25SEP2017)	**8.2.2.2**	**Lung Function: Removed sentence stating PEEP and tidal volume will be assessed for invasive ventilation only. Added Table 8-2 Normal ranges for Lung Function parameters Added clarification that parameters are measured daily**
3.0 (25SEP2017)	**8.2.2.2**	**Mechanical Ventilation: Added +1 day to calculation for Time to being off ventilator.**
3.0 (25SEP2017)	**8.2.2.2**	**Deaths during 90 day study period: Added + 1 day to calculation for Time to death.**
3.0 (25SEP2017)	**8.2.2.2**	**Purine data: Section added**
3.0 (25SEP2017)	**8.3**	**Updated text for discharge from hospital for Day 1 to 28 and Day 1 to 90 and similarly for ICU. Added +1 day to calculation for Time to discharge from Hospital/ICU.**
3.0 (25SEP2017)	**8.3**	**Exogenous Creatinine Clearance section added**
3.0 (25SEP2017)	**9.1.6**	**Deaths during follow-up will be included in summaries and listings.**
3.0 (25SEP2017)	**9.2.2**	**Added normal ranges for pH.**
3.0 (25SEP2107)	**9.2.3**	**Corrected typo in 'performed'**
3.0 (25SEP2017)	**11**	**Reverted numbering back to original Interim analysis numbering**
3.0 (25SEP2017)	**14**	**Added references for lab conversions**
3.1 (27 SEP2017)		**27SEPT2017: Client sign off received on version 3.0 with request that two minor changes in wording were made as documented below. Client email confirmation received that they do not require client sign-off on version with minor changes.**

Version (date)	Section	Summary of change
3.1 (27SEP2017)	**8.1.5** **8.3**	**Following minor updates done after sign off at client request:** **1) 8.1.5 Wording on microbial infection categories:** **a. Text regarding manual review removed** **b. Text in parenthesis following category 'Unknown' to be removed as text was only to clarify for the purposes of processing the data.** **2) 8.3 Discharge from ICU/Intermediate care for Day 1 to Day 28 and Day 1 to day 90** **a. Ordering of wording changed from "died whilst still in ICU prior to Day 28" to "died prior to Day 28 whilst still in ICU"** **3) 8.3 Discharge from Hospital for Day 1 to Day 28 and Day 1 to Day 90. Ordering of wording changed as above.**

14. References

Bauer P, Köhne K. Evaluation of experiments with adaptive interim analyses. Biometrics. 1994;50(4):1029-41.

Chawla LS, Amdur RL, Amodeo S, et al. The severity of acute kidney injury predicts progression to chronic kidney disease. Kidney Int. 2011;79(12):1361-9.

Gaspari F, Perico N, Ruggenenti, P, et al. Plasma clearance of nonradioactive iohexol as a measure of glomerular filtration rate. J Am Soc Nephrol. 1995;6: 257-263.

https://www.niddk.nih.gov/health-information/health-communication-programs/nkdep/lab-evaluation/gfr-calculators/adults-conventional-unit-ckd-epi/Pages/default.aspx)

https://www.klinischediagnostiek.nl/artikelen/bloedgassen#5_referentiewaarden based on https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1114160/

Oppert M, Engel C, Brunkhorst FM, et al. Acute renal failure in patients with severe sepsis and septic shock--a significant independent risk factor for mortality: results from the German Prevalence Study. Nephrol Dial Transplant. 2008;23(3):904-9.

Vaara ST, Korhonen AM, Kaukonen KM, et al. Fluid overload is associated with an increased risk for 90-day mortality in critically ill patients with renal replacement therapy: data from the prospective FINNAKI study. Crit Care. 2012;16(5):R197.

15. Appendices

15.1. Schedule of Study Procedures

Table 15-1 Schedule of Assessments

Assessment	Screening (≤ 24 h)	Baseline (≤ 24 h)	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7 (± 1 day)	Day 14 (± 2 days)	Day 21 (± 3 days)	Day 28 (± 3 days)	Day 60 (± 5 days)	Day 90 (± 10 days)
AKI diagnosis[a] (pre-screening) Record site of infection and pathogen	X													
Inclusion and exclusion criteria	X	X[b]												
Informed consent	X													
Medical history	X													
Demographics	X													
Child-Pugh score[c]	X													
Recent hematology and clinical chemistry results, if available	X													
Recent microbial test results, if available	X													
Pregnancy test (urine or blood)[d]	X													

Assessment	**Screening (≤ 24 h)**	**Baseline (≤ 24 h)**	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7 (± 1 day)**	**Day 14 (± 2 days)**	**Day 21 (± 3 days)**	**Day 28 (± 3 days)**	**Day 60 (± 5 days)**	**Day 90 (± 10 days)**
Local laboratory confirmatory serum creatinine sample[e], or confirmatory assessment of continuation of decreased urine output	X													
Randomization[f]		X												
Vital signs (BP, HR, OS, RR, T)[g]		X	X[h]	X[h]	X[h]	X	X	X	X	X	X	X		
Physical examination		X	X	X	X	X	X	X	X	X	X	X		
APACHE II score		X												
SAPS-2 score		X												
SOFA score[i]		X	X	X	X	X	X	X	X	X	X	X		
EQ-5D**Error! Reference source not found.**		X												X
Alkaline phosphatase		X												
Time from first diagnosis of SA-AKI to start of recAP treatment		X												
Treatment			X	X[k]	X[k]									

Assessment	Screening (≤ 24 h)	Baseline (≤ 24 h)	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7 (± 1 day)	Day 14 (± 2 days)	Day 21 (± 3 days)	Day 28 (± 3 days)	Day 60 (± 5 days)	Day 90 (± 10 days)
Arterial partial pressure of O_2 (in ICU or intermediate care unit only) for mechanically ventilated patients		X	X	X	X	X	X	X	X	X	X	X		
Blood: serum creatinine and BUN[l]		X[e]	X	X	X	X	X	X	X	X	X	X	X	X
Urine (6 ± 1 h collection) creatinine, BUN[m]		X[n]	X	X	X	X	X	X	X	X	X	X		
Volume of urine[o]		X[p]	X	X	X	X	X	X	X	X	X	X		
Pharmacokinetics[q]			X	X	X	X	X	X	X					
ECG (12-lead)[r]		X			X					X				
Hematology (Hgb, Hct, leukocytes, diff leukocytes, erythrocytes, thrombocytes, and APTT)[d]		X	X		X		X		X	X	X	X		
Clinical chemistry (CRP, ALT, AST, GGT, urea, LDH, creatinine, bilirubin, CPK, total protein, albumin, glucose, sodium, potassium, chloride, bicarbonate, and lactate)[d]		X	X		X		X		X	X	X	X		

Assessment	**Screening (≤ 24 h)**	**Baseline (≤ 24 h)**	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7 (± 1 day)**	**Day 14 (± 2 days)**	**Day 21 (± 3 days)**	**Day 28 (± 3 days)**	**Day 60 (± 5 days)**	**Day 90 (± 10 days)**
Biomarkers for systemic inflammation (e.g., IL-6, CRP, LBP)[s]		X	X	X	X	X	X	X	X	X	X	X		
Serology (IgG, IgE, and total immunoglobulin)[s]		X								X		X		
Anti-drug antibodies		X								X		X	X[t]	X[t]
Tubular injury biomarkers (e.g., KIM-1, IL-18, GST-alpha) in urine[s]		X	X	X	X	X	X	X	X	X	X	X		
Kidney function markers (urine creatinine, BUN/urea clearance, fractional excretion of urea and urine output)[s]		X	X	X	X	X	X	X	X	X	X	X		
Kidney function markers (sodium and fractional excretion of sodium)[s]		X	X	X	X	X	X	X	X					
Kidney function markers (serum creatinine and proteinuria)[s]		X	X	X	X	X	X	X	X	X	X	X	X	X
Concomitant medication	X[u]	X	X	X	X	X	X	X	X	X	X	X		
Patient on RRT, and start or stop date			X	X	X	X	X	X	X	X	X	X		

Assessment	Screening (≤ 24 h)	Baseline (≤ 24 h)	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7 (± 1 day)	Day 14 (± 2 days)	Day 21 (± 3 days)	Day 28 (± 3 days)	Day 60 (± 5 days)	Day 90 (± 10 days)
Need for dialysis dependency													X	X
Name, start or stop date, and dose of vasopressor and inotropic therapy[v]		X	X	X	X	X	X	X	X	X	X	X		
Mechanical ventilation and lung function[w] (start or stop date, FiO_2, PEEP, tidal volume, P/F ratio), ventilated patients only		X	X	X	X	X	X	X	X	X	X	X		
Adverse events	X	X	X											
Mortality	X	X	X											
Discharge from ICU or intermediate care unit / admission or discharge from hospital[Error! Reference source not found.]	X	X	X											

Abbreviations: AKI = acute kidney injury; ALT = alanine aminotransferase; APACHE = acute physiology and chronic health evaluation; APTT = activated partial thromboplastin time; AST = aspartate aminotransferase; BP = blood pressure; BUN = blood urea nitrogen; CPK = creatine phosphokinase; CRP = c-reactive protein; diff = differential; ECG = electrocardiogram; FiO2 = fraction of inspired oxygen; GGT = gamma-glutamyl transpeptidase; GST-alpha = alpha-glutathione s-transferase; h = hour; Hct = hematocrit; Hgb = hemoglobin; HR = heart rate; ICU = intensive care unit; IgE = immunoglobulin E; IgG = immunoglobulin G; IL-6 = interleukin-6; IL-18 = interleukin-18; KIM-1 = kidney injury molecule-1; LBP = lipopolysaccharide binding protein; LDH = lactate dehydrogenase; OS = oxygen saturation; PEEP = positive end expiratory pressure; P/F ratio = fraction PaO_2/FiO_2; RR = respiratory rate; RRT = renal replacement therapy; SAPS-2 = simplified acute physiology score 2; SOFA = sequential organ failure assessment; T = temperature.

[y.] The AKI diagnosis can be made according to one of the AKIN serum creatinine criteria (absolute or relative increase, see inclusion criteria **Error! Reference source not found.** and **Error! Reference source not found.**), or according to the AKIN urine output criteria (urinary output < 0.5 mL/kg/h for > 6 hours, see inclusion criterion **Error! Reference source not found.**), the oliguria or anuria should still meet the AKIN urine output criteria prior to randomization and study drug administration.

[z.] Confirmatory.

aa. Only for patients with liver disease.

bb. Local laboratory.

cc. See flowchart (Section **Error! Reference source not found.**, **Error! Reference source not found.**) for options and preference for reference serum creatinine value. The reference creatinine value is the serum creatinine value according to the following order of preference: 1) lowest value within 3 months of the hospital admission. If not available, 2) at hospital admission. If not available, 3) at ICU or intermediate care unit admission. If not available, 4) lowest value between 3 and 12 months prior to hospital admission.

dd. When the diagnosis of AKI is made according to one of the AKIN serum creatinine criteria (absolute or relative increase, see inclusion criteria **Error! Reference source not found.** and **Error! Reference source not found.**), patients will be eligible for the study and can be randomly assigned when the volume-corrected serum creatinine sample, taken at screening confirms the continuation of AKI according to the AKIN criteria for serum creatinine. When the AKI diagnosis was made according to the AKIN urine output criteria (urinary output < 0.5 mL/kg/h for > 6 hours, see inclusion criterion **Error! Reference source not found.**), the oliguria or anuria should still meet the AKIN urine output criteria prior to randomization and study drug administration.

ee. Vital signs in ambulant patients will be obtained with the patient in a sitting position and after 5 minutes rest. Blood pressure will be monitored non-invasively. In patients who already have an arterial line placed as part of standard or care, readings from invasive blood pressure monitoring are to be recorded.

ff. Additionally, vital signs (excluding temperature) will also be monitored during study drug infusion on all treatment days at the following times: a) immediately before the administration of the study drug, b) within 5 minutes of the start of the study drug infusion, c) 30 minutes after the start of the study drug infusion, d) immediately after the completion of the administration of the study drug, which includes post-dose saline flushing, e) 30 and 60 minutes after completion of study drug administration, f) 2 hours, 3 hours, 4 hours, and 5 hours after completion of study drug administration (Day 1 only).

gg. SOFA score to be obtained on each visit day as long as the patient is in the ICU or intermediate care unit, and at discharge from ICU or intermediate care unit .

hh. EQ-5D will be performed at baseline, at discharge from the ICU or intermediate care unit, and at the Day 90 visit. In case the patient is unconscious, EQ-5D questionnaire will be completed by a next of kin.

ii. At 24 ± 1 hour after the previous drug administration.

jj. Creatinine and BUN will be measured by a central laboratory. At Days 60 and 90, only serum creatinine will be measured. When patients have a Foley catheter, serum creatinine samples should be collected prior to and immediately after each urine collection for at least up to Day 7. If the patient is discharged from the ICU or intermediate care unit , the Foley catheter might be removed. In this case, a patient might urinate spontaneously and all efforts should be undertaken to start collecting urine produced from this time point onward. Approximately 6 hours later (exact duration needs to be recorded) the patient might urinate again and this urine will be used for analysis, and a blood sample will be drawn at this time too. The urine volume produced over approximately 6 hours will be entered in the eCRF.

kk. Urine creatinine and urea will be measured by a central laboratory. The central laboratory will calculate blood urea nitrogen (BUN) clearance at all visits from Day 1 to Day 7, inclusive, and on subsequent visit days if reliable urine collection is possible. Urine will be collected within a 6 ± 1 hour period at all visits from Day 1 to Day 28, only if reliable urine collection is possible (i.e., patient remains in the ICU or intermediate care unit or hospital).

ll. These assessments will be performed before treatment if possible. Treatment should not be delayed because of these assessments.

mm. Urine volume collection in a 6 ± 1 hour collection period, only if reliable urine collection is possible (i.e., patient remains in the ICU or intermediate care unit or hospital). The volume should be corrected to account for the volume of samples previously taken from the total urine initially collected.

nn. Only when possible within the 24-hour time window from first AKI diagnosis to treatment.

oo. Assays will be performed by a central reference laboratory. See Section **Error! Reference source not found.** for sampling details.

pp. A 12-lead ECG with at least 30-second rhythm strip will be recorded after the patient has rested supine or semi-recumbent for at least 5 minutes.

qq. Central reference laboratory.

rr. Samples taken on Days 60 and 90 will only be analyzed in case of a positive result on Day 28.

ss. Verification that no concomitant medications that should be avoided are taken.

tt. The actual stop date is collected for calculation of shock-free days. Only required when the patient is in the ICU or intermediate care unit.

uu. Daily, as long as the patient requires mechanical ventilation. As appropriate, record start and stop dates and times of mechanical ventilation, including the settings required and the O_2 in the blood.

vv. Actual admission dates and both planned and actual discharge dates from the ICU or intermediate care unit and hospital (planned meaning when decision is taken to discharge the patient, not necessarily being the same as the actual discharge date, e.g., because of lack of beds on the regular ward).

15.2. Schedule of DMC Reviews

Table 15-2 Schedule of DMC Reviews

		Milestone *
Part 1	**Teleconference**	75 patients (60%)
	Face to face	IA-120 patients
Part 1-Electronic Reviews		25 patients
		50 patients
		100 patients
Part 1 – Ad hoc Review		Between 90 and 120 Patients
Part 2	**Teleconference**	60 additional patients in Part 2 patients (at least 180 total)
	Teleconference	125 additional patients in Part 2 patients (at least 245 total)
Part 2-Electronic Reviews		30 additional patients in Part 2 patients (at least 150 total)
		90 additional patients in Part 2 patients (at least 210 total)
Part 2-Ad hoc Review		Between 125 and 170 patients in Part 2 (at least 245-290 total)

* Milestone refers to patients with at least 7 days of laboratory data, allowing for patients that were randomized but discontinued/died prior to 7 days

15.3. Weight Ranges and Pre-calculated Corresponding Volumes

Table 15-3 Weight Ranges and Pre-calculated Corresponding Volumes

Body weight (kg)	Body weight (lb)	Volume drawn from 4 vials (mL)	Volume discarded from syringe (mL)	IMP retained in syringe (mL)	Saline added to reconstitute (mL)	Total volume in syringe (mL)
35 < 40	77 < 88	20	12	8	42	50
40 < 45	88 < 99	20	11	9	41	50
45 < 50	99 < 110	20	10	10	40	50
50 < 55	110 < 121	20	9	11	39	50
55 < 60	121 < 132	20	8	12	38	50
60 < 65	132 < 143	20	7	13	37	50
65 < 70	143 < 154	20	6	14	36	50
70 < 75	154 < 165	20	5	15	35	50
75 < 80	165 < 176	20	4	16	34	50
80 < 85	176 < 187	20	3	17	33	50
85 < 90	187 < 198	20	2	18	32	50
90 < 95	198 < 209	20	1	19	31	50
95 - 115	209 < 253	20	0	20	30	50

Abbreviation: I

IMP = investigational medical product.

In line with Table 15-3, the volume of investigational product (mL) administered to each patient is defined for a range of body weights – hence, the dose (mg/kg) can vary slightly dependent on where a patient lies within a weight range, even if the infusion is prepared correctly. The investigation product has a concentration of 8 mg/mL, thus the exact dose is calculated as

$$\frac{5 \times \text{IMP retained in syringe (mL)} \times \text{Treatment group}}{\text{Weight (kg)}}$$

where Treatment group = 0.4, 0.8, and 1.6 respectively for the recAP groups. The minimum doses for a correctly prepared infusion (corresponding to the maximum weight within each range in Table 15-3) are 0.4 mg/kg, 0.8 mg/kg and 1.6 mg/kg respectively for each of the recAP treatment groups. The maximum doses (corresponding to a patient with body weight 35 kg) are 0.457 mg/kg, 0.914 mg/kg and 1.829 mg/kg respectively.

Therefore, the dose windows in Section 4 used to assign patients to received treatment group based on average dose are based on the average midpoints between the minimum and maximum possible doses of each weight group for each treatment group (0.62 mg/kg for the split between the 0.4 mg/kg and 0.8 mg/kg groups; and 1.23 mg/kg as the cutoff between the 0.8 mg/kg and 1.6 mg/kg groups).

15.4. GFR formula

Based on this CKD-EPI formula the glomerular filtration rate (eGFR) is calculated based on the serum creatinine value as:

$GFR = 141 \times \min(S_{cr}/\kappa, 1)^{\alpha} \times \max(S_{cr}/\kappa, 1)^{-1.209} \times 0.993^{Age} \times 1.018$ [if female] × 1.159 [if black]

where:
S_{cr} is serum creatinine in mg/dL,
κ is 0.7 for females and 0.9 for males,
α is -0.329 for females and -0.411 for males,
min indicates the minimum of S_{cr}/κ or 1, and
max indicates the maximum of S_{cr}/κ or 1.