CLINICAL STUDY PROTOCOL
EUDRACT Number 2014-000761-40
IND number: 117605
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

PROTOCOL NO.
AP-recAP-AKI-02-01

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Version of Protocol: Protocol Version 3.0, including Amendment 2

Date of Protocol Amendment: 03 Feb 2016

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CONFIDENTIAL

All financial and non-financial support for this study will be provided by AM-Pharma B.V. The concepts and information contained in this document or generated during the study are considered proprietary and may not be disclosed in whole or in part without the expressed, written consent of AM-Pharma B.V.

The study will be performed in accordance with the ethical principles that have their origin in the Declaration of Helsinki, the International Council for Harmonisation ICH E6(R1), US Code of Federal Regulations, and all other applicable regulations.

Protocol Approval – Sponsor Signatory

Study Title 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

Protocol Number AP-recAP-AKI-02-01

Protocol Date 03 Feb 2016

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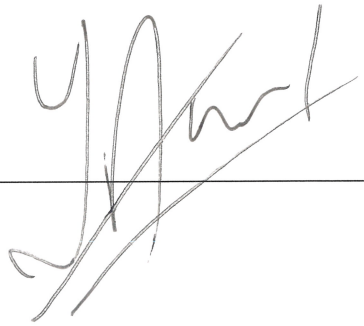

Signature

- 5 FEB. 2016

Date

Protocol Approval – Principal/Coordinating Investigator

Study Title	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
Protocol Number	AP-recAP-AKI-02-01
Protocol Date	03 Feb 2016

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February 4th 2016

Signature Date

Declaration of Investigator

I have read and understood all sections of the protocol titled "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" and the accompanying investigator's brochure, version 2.1, dated 09 Oct 2014.

I agree to supervise all aspects of the protocol and to conduct the clinical investigation in accordance with the final protocol version 3.0, including amendment 2, dated 03 Feb 2016, the International Council for Harmonisation (ICH) harmonised tripartite guideline E6(R1): Good Clinical Practice and all applicable government regulations. I will not make changes to the protocol before consulting with AM-Pharma B.V. or implement protocol changes without independent ethics committee approval except to eliminate an immediate risk to patients. I agree to administer study drug only to patients under my personal supervision or the supervision of a sub-investigator.

I will not supply the investigational drug to any person not authorized to receive it. Confidentiality will be protected. Patient identity will not be disclosed to third parties or appear in any study reports or publications.

I will not disclose information regarding this clinical investigation or publish results of the investigation without authorization from AM-Pharma B.V.

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Signature of Investigator Date

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Printed Name of Investigator

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Protocol Synopsis

Protocol Number:	AP-recAP-AKI-02-01
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Study Phase:	2a/2b
Study Sites:	Approximately 100 sites will participate in the study; approximately 75 in the European Union and approximately 25 in the United States and Canada. Recruitment will continue during the interim analysis.
Indication:	Sepsis-associated acute kidney injury (SA-AKI)
Rationale:	As there are no guidelines for the development of drugs for the indication SA-AKI, the proposed design was determined to be optimal by a group of leading global experts in acute kidney injury (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 the 120th patient of Part 1 has completed the Day 7 visit. The 3 active doses 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 1 healthy volunteer study with recombinant human AP (recAP). Assuming comparable safety profiles, the optimal dose will be selected at the interim analysis by an independent data monitoring committee (DMC). The primary endpoint, area under the time-corrected endogenous creatinine clearance curve, 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 the first 120 patients from Part 1 only, 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 chronic kidney disease [CKD]).

Objectives:

Primary objectives

- 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.

Secondary objectives

- To investigate the safety and tolerability of recAP in patients with SA-AKI.
- To investigate the pharmacokinetics (PK) of recAP in a subset of patients with SA-AKI (in the first 120 patients from Part 1 only).
- 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).

Other objectives

To determine whether specific patient groups that 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:
  - Kidney function markers (e.g., urine and serum creatinine, BUN/urea clearance, sodium, proteinuria, fractional excretion of sodium and urea, and urine output)
  - Tubular injury biomarkers (e.g., kidney injury molecule-1 [KIM-1], interleukin 18 [IL-18],

alpha-glutathione s-transferase [GST-alpha])
  - Biomarkers for systemic inflammation (e.g., IL-6, C-reactive protein [CRP], lipopolysaccharide-binding protein [LBP])
  - Glomerular filtration rate (eGFR by chronic kidney disease epidemiology collaboration [CKD-EPI])
  - APACHE II score (<25, ≥25)
- Timing from first diagnosis of SA-AKI to start of recAP treatment, e.g., in time intervals (0 to < 6 hours, 6 to < 12 hours, 12 to < 18 hours, 18 to < 24 hours)
- Baseline Acute Kidney Injury Network (AKIN) stage (stage 1, stage 2, stage 3)

Additional parameters may also be considered (e.g., urine output [over or under 200 mL/day], diabetes mellitus [yes or no], hypertension [yes or no], age [< 55 years old, ≥ 55 years old and < 70 years old, or ≥ 70 years old], sex (male or female), risk scores [high or low]).

Patient Population:

Adult (18 to 85 years, inclusive) patients admitted to the intensive care unit (ICU) or intermediate care unit for whom a diagnosis of sepsis (according to criteria defined by the American College of Chest Physicians/Society of Critical Care Medicine) can be made, and who also show signs of early AKI according to the AKIN criteria.

Patients with life support limitations, already on dialysis (renal replacement therapy [RRT]) or a decision has been made to initiate RRT within 24 hours after planned start of study drug administration, with known prior history of CKD with a documented eGFR of < 60 mL/min or a known history of persistent creatinine level equal or greater than 150 µmol/L, or with other causes of AKI present, will be excluded.

Study Design:

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

A minimum of 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 continued 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 with an estimated 50 patients during the interim analysis. Patients enrolled during Part 1 and during the interim analysis will be randomly assigned to receive, by 1-hour intravenous (IV)

infusion, either placebo (Part 1; $n_1 = 30$) or one of 3 different doses of recAP (Part 1; $n_1 = 30$ 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 by 1-hour IV infusion once daily for 3 days (Days 1, 2, and 3). The interim analysis on the primary endpoint will be performed on all data collected after the 120th patient of Part 1 has completed the Day 7 visit of the study to select the dose to be administered in Part 2. The dose chosen will be the 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. In Part 2, patients will be randomly assigned to receive, by 1-hour IV infusion, either placebo ($n_2 = 85$) or the dose of recAP ($n_2 = 85$) selected during the interim analysis. Patients recruited during the interim analysis period to the dose selected in Part 2 will form part of the Part 2 populations, but those recruited to the doses that are not selected will be included in the Part 1 population.

Each part involves the following schedule of events: potential patients who have been admitted to the ICU or intermediate care unit will undergo a pre-screening to assess the presence of SA-AKI, will provide informed consent, and will undergo screening assessments to determine eligibility. As soon as possible when all inclusion and none of the 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 first diagnosis of sepsis.

Due to potential unblinding, it is not allowed to locally measure alkaline phosphatase (AP) activity in the blood up to Day 14 (inclusive) irrespective of whether the patient is in the ICU or intermediate care unit or in the ward.

Estimated Study Duration:

Duration of the study for a patient is defined from the date that signed written informed consent is provided until the last follow-up visit on Day 90.

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

Efficacy Assessments:

The following assessments will be considered as efficacy measurements:

- Urine volume, urinary creatinine, and the average of two serum creatinine measurements will be used for calculation of the time-corrected endogenous creatinine clearance at all visits from baseline to Day 7. Calculations will be performed by the central laboratory. The measurements from Day 1 to Day 7, inclusive, will be used to calculate the primary endpoint. Only if reliable urine collection is possible, urine will be collected on Days 14, 21, and 28 for time-corrected endogenous creatinine clearance calculation (i.e., patient is in the ICU or intermediate care unit or hospital). If reliable urine collection is not possible, one serum creatinine sample still needs to be taken for calculating eGFR.
- Urine volume collection in a 6 ± 1 hour collection period at baseline and at all visits from Day 1 to Day 28, but only required if reliable urine collection is possible (i.e., patient is in the ICU or intermediate care unit or hospital)
- Blood urea nitrogen (BUN) clearance at all visits from baseline to Day 7
- Urinary urea at baseline and at all visits from Day 1 to Day 28, only if reliable urine collection is possible (i.e., patient is in the ICU or intermediate care unit or hospital)
- Fractional excretion of sodium at all visits from baseline to Day 7
- Fractional excretion of urea at all visits from baseline to Day 7
- Proteinuria at baseline and at all visits from Day 1 to Day 90
- Tubular injury biomarkers, KIM-1, IL-18, and GST-alpha, in urine at baseline and at all visits from Day 1 to Day 28
- Serum creatinine at baseline and at all visits from Day 1 to Day 90

- Serum BUN at baseline and at all visits from Day 1 to Day 28
- Systemic inflammatory serum biomarkers, e.g., IL-6, CRP, LBP, at baseline and at all visits from Day 1 to Day 28
- RRT incidence, considered as the key secondary endpoint, during the study period Day 1 to Day 28
- RRT duration, during the study period Day 1 to Day 28
- Incidence of dialysis dependency on Day 60 and Day 90
- Sequential Organ Failure Assessment score results at baseline, at all visits from Day 1 to Day 28 (when patient is in the ICU or intermediate care unit), and on the day of ICU or intermediate care unit discharge if discharged on a non-visit day. Note that platelets, bilirubin, creatinine, and urine output are required to obtain a SOFA score.
- Quality of life, EQ-5D questionnaire at baseline, at ICU or intermediate care unit discharge, and Day 90
- Recording start and stop date, name, and mean daily dose of vasopressor and inotropic therapy for determination of shock-free days, at baseline and at all visits from Day 1 to Day 28, only required when patient is in the ICU or intermediate care unit
- Length of hospital and ICU or intermediate care unit stay by recording 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)

Safety Assessments: The following assessments will be considered safety measurements:

- Vital signs, including blood pressure, heart rate, oxygen saturation, respiratory rate, and body temperature at baseline and at all visits from Day 1 to Day 28. During the 3 dosing days (Day 1 to Day 3), repeated vital sign measurements will be performed.
- Physical examination at baseline and at all visits from Day 1 to Day 28
- 12-lead ECG at baseline and at visits on Days 3 and 14
- Local laboratory at baseline and at visits on Days 1, 3, 5, 7, 14, 21, and 28:

- Hematology
- Clinical chemistry, including liver function parameters (aspartate transaminase, alanine aminotransferase, gamma-glutamyl transpeptidase lactate dehydrogenase, and bilirubin; excluding AP)

- Serology (IgG, IgE and total immunoglobulin) at baseline and at visits on Days 14 and 28
- Anti-drug antibodies at baseline and at visits on Days 14, 28, 60, and 90. Samples taken on Days 60 and 90 will only be analyzed in case of a positive result on Day 28
- Concomitant medication at screening, baseline, and all visits from Day 1 to Day 28
- AEs during the duration of the study
- Mortality (monitored continuously throughout the study)

Exploratory Assessments:

The following assessments will be considered as exploratory measurements:

- PK analyses performed at all visits from Day 1 to Day 7 (inclusive) in the first 120 patients from Part 1 only
- Lung function. The following must be recorded during the study:
  - Ventilation modality (e.g., non-rebreathing mask, non-invasive mechanical ventilation, invasive mechanical ventilation) and start and stop dates
  - Non-mechanical ventilation: non-rebreathing mask, or nasal oxygen delivery (liters of oxygen/min, estimated daily average)
  - Mechanical ventilation (non-invasive and invasive):
    - Arterial partial oxygen pressure (PaO_2) (estimated daily average)
    - Fraction of inspired oxygen (FiO_2) and positive end-expiratory pressure (PEEP) (estimated daily average)
    - Tidal volume (estimated daily average) for invasive mechanical ventilation only)
    - To enable calculation of the fraction PaO_2/FiO_2: PaO_2 and FiO_2 must be captured at the same time point. Only one simultaneous measurement will be used. In case of multiple measurements of this fraction the worst value must be used.

Study Drug, Dosage, and Route of Administration:	Study drug will be administered by 1-hour IV infusion as soon as possible on Day 1, and on Days 2 and 3 at 24 ± 1 hour after the previous drug administration, by trained staff in the ICU or intermediate care unit. Patients randomly assigned to receive recAP in Part 1 or during interim analysis will receive one of the following 3 doses of recAP: 0.4 mg/kg (250 U/kg), 0.8 mg/kg (500 U/kg), or 1.6 mg/kg (1000 U/kg). Patients will receive study drug by 1-hour IV infusion once daily for 3 days (Days 1, 2, and 3). At the start of each drug administration, the exact volume of recAP or placebo to be administered to each patient will be calculated on the basis of the patient's weight. Patients weighing between 95 to 115 kg will receive the same dose as that for patients weighing 100 kg. A medication preparation instruction sheet including a table with weight ranges and pre-calculated corresponding volumes will be provided. The maximum weight is limited to 115 kg (253 lb). The volume of the matching placebo medication will be identical to the volume the patient would receive if randomly assigned to recAP.

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 the optimal recAP dose and placebo treatment groups in Part 2 (for a total sample size of n = 290 patients) is planned. Custom programmed simulations were performed using SAS® software 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.

Statistical Methods:

Statistical analyses will be performed using SAS software Version 9.2 or later. Continuous variables will be summarized using descriptive statistics. Categorical variables will be summarized using frequency counts and percentages. All data collected across the 4 treatment groups throughout the study will be listed in data listings.

All statistical tests will be 2-sided and performed using a 5% significance level, leading to 95% (2-sided) confidence intervals (CIs). 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. 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 primary efficacy endpoint, area under the time-corrected endogenous creatinine clearance curve from Day 1 to Day 7 (AUC_{1-7}), will be summarized using descriptive statistics and analyzed using analysis of variance with treatment and site as explanatory variables. The analysis will be performed separately for Parts 1 and 2: for Part 1, AUC_{1-7} will be compared between the 3 recAP doses and placebo. This analysis will be considered in conjunction with the safety data to determine the optimal recAP dose for use in Part 2. For Part 2, the optimal recAP dose will be compared with 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 level α if it and all intersection hypotheses involving it are all rejected at local level α. The testing strategy used to combine results from Parts 1 and 2 will be a combination test based on the inverse normal method. The difference in least squares means between the treatment groups will also be presented along with the associated 95% CI.

Analyses of the secondary efficacy endpoints will be conducted at the conclusion of Part 2.

The key secondary endpoint, the incidence of RRT from Day 1 to Day 28, inclusive, will be summarized by treatment group using counts and percentages. Should a statistically significant result be obtained from the combination test analysis of the primary endpoint, the optimal recAP dose will be formally compared with placebo using a logistic regression model with treatment group and site as explanatory variables. Otherwise the results will be reported as exploratory analyses only. The odds ratio and associated 95% CI from this comparison will also be presented.

The safety summaries will include all 4 treatment groups, comprising all patients in the safety set.

Date of Protocol: 03 Feb 2016

List of Abbreviations

Abbreviation	Definition
ADP	adenosine diphosphate
AE	adverse event
AKI	acute kidney injury
AKIN	Acute Kidney Injury Network
ALT	alanine aminotransferase
AMP	adenosine monophosphate
ANOVA	analysis of variance
AP	alkaline phosphatase
APACHE	Acute Physiology and Chronic Health Evaluation II
AST	aspartate aminotransferase
ATP	adenosine triphosphate
$AUC_{0\text{-}inf}$	area under the concentration-time curve from zero up to an infinite time
$AUC_{1\text{-}7}$	area under the time-corrected endogenous creatinine clearance curve from Day 1 to Day 7
BiAP	bovine intestinal alkaline phosphatase
BUN	blood urea nitrogen
cAMP	cyclic adenosine monophosphate
CFR	Code of Federal Regulations
CI	confidence interval
$Cl_{Iohexol}$	iohexol clearance
CKD	chronic kidney disease
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration
C_{max}	observed maximum plasma or serum concentration after administration
C_{min}	observed minimum plasma or serum concentration after administration
CPAP	continuous positive airway pressure
CRP	C-reactive protein
DMC	data monitoring committee
ECG	electrocardiogram
eCRF	electronic case report form
eGFR	estimated glomerular filtration rate
EQ-5D	EuroQol-5D

Abbreviation	Definition
ERPF	effective renal plasma flow
FDA	Food and Drug Administration
FiO_2	fraction of inspired oxygen
GFR	glomerular filtration rate
GGT	gamma-glutamyl transpeptidase
GST-alpha	alpha-glutathione s-transferase
hCG	human chorionic gonadotropin
ICF	informed consent form
ICH	The International Council for Harmonisation
ICU	intensive care unit
IEC	independent ethics committee
IgE	immunoglobulin E
IgG	immunoglobulin G
IL-6	interleukin 6
IL-18	interleukin 18
IRB	institutional review board
ITT	intent to treat
IV	Intravenous
IVRS	interactive voice response system
KDIGO	Kidney Disease: Improving Global Outcomes
KIM-1	kidney injury molecule-1
LBP	lipopolysaccharide-binding protein
LDH	lactate dehydrogenase
LOCF	last observation carried forward
LPS	lipopolysaccharide
MDRD	Modification of Diet in Renal Disease
MedDRA	Medical Dictionary for Regulatory Activities
NSAID	non-steroidal anti-inflammatory drug
PD	pharmacodynamic(s)
PEEP	positive end expiratory pressure
P/F ratio	fraction PaO_2/FiO_2 (Carrico index)
PK	pharmacokinetic(s)

Abbreviation	Definition
PP	per protocol
recAP	recombinant human alkaline phosphatase
RRT	renal replacement therapy
SA-AKI	sepsis-associated acute kidney injury
SAE	serious adverse event
SAP	statistical analysis plan
SAPS-2	Simplified Acute Physiology Score 2
SIRS	systemic inflammatory response syndrome
SOFA	Sequential Organ Failure Assessment
SPM	study procedures manual
TEAE	treatment-emergent adverse event
TLR4	toll-like receptor 4

1 Introduction

Sepsis is a major cause of multiple organ dysfunction and death in the intensive care unit (ICU). Despite modern anti-microbial therapy and intensive care facilities, the mortality rate of sepsis remains high (Martin et al 2003). It is estimated that in the United States alone, sepsis occurs in 750 000 patients per year with a mortality rate of approximately 30% to 50% (CDC 1990; Rangel-Frausto et al 1995; Angus et al 2000; Annane et al 2000). In addition, sepsis is the most common cause of non-cardiac death in the ICU (Parrillo et al 1990). Sepsis is a systemic inflammatory cascade that occurs in the human body in response to an infection with bacteria, or other pathogens (Rackow 1986; Bone et al 1997). Patients with sepsis present with clinical manifestations of systemic inflammation and suspicion or evidence of infection.

Sepsis-associated acute kidney injury (SA-AKI) is a serious condition. Sepsis-associated acute kidney injury results in 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). Acute kidney injury is thought to be a multi-factorial disease with inflammatory, direct nephrotoxic, and ischemic insults acting simultaneously with other pathogenic responses to rapidly cause functional failure of the kidney (Bonventre and Yang 2011; Wen et al 2011; Gomez et al 2014). In sepsis, the initial host response to an infection, mostly caused by bacteria, becomes amplified and then deregulated, bringing the body into an inflammatory state (Cohen 2002). There are no pharmacological interventions approved for the treatment of SA-AKI. Currently, renal replacement therapy (RRT) is the only supportive treatment option available for AKI (Vincent et al 2006; Oppert et al 2008; Bagshaw et al 2009).

Alkaline phosphatase (AP) is an endogenous enzyme present in many cells and organs (e.g., intestines, placenta, liver, bone, kidney, and granulocytes) that exerts detoxifying effects through dephosphorylation of endotoxins (Bentala et al 2002; Koyama et al 2002) and other pro-inflammatory compounds including extracellular adenosine triphosphate (ATP) (Picher et al 2003). Local AP concentrations reflect the host defense against endotoxin in the kidney (Kapojos et al 2003), and during ischemia enzyme levels are markedly depleted, associated with the development of AKI (Khundmiri et al 1997). Apart from local effects in the kidney, AP may attenuate the innate immune response, as dephosphorylation of endotoxin abolishes its biological activity and acts as a toll-like receptor 4 (TLR4) antagonist (Wy et al 2000). In

animal models of sepsis, AP administration attenuates the inflammatory response and reduces mortality (Beumer et al 2003; Su et al 2006). There is increasing evidence that the protein plays a significant role in host defense and innate immunity, particularly against inflammatory reactions due to lipopolysaccharide (LPS) release (Verweij et al 2004; Su et al 2006).

Lipopolysaccharides (also named endotoxins) are constituents of the cell wall of gram-negative bacteria, which can also be present in the circulation during gram-positive infections (Marshall et al 2004), and are released when these bacteria disintegrate. It is a group of negatively charged molecules, of which the Lipid A moiety binds through 2, for this purpose essential, phosphate groups to its receptor, the CD14-TLR4 complex. These receptors are present on the surface of leukocytes (macrophages, white blood cells) and endothelial cells, and once activated they secrete a number of inflammatory cytokines. These in turn can cause a devastating and life-threatening derailment of the human innate immune system.

Alkaline phosphatase is thought to exert a dual mode of action. First of all, it binds and subsequently dephosphorylates LPS (Kiffer-Moreira et al 2014), thereby eliminating the root cause of the systemic inflammatory response syndrome (SIRS). Secondly, the enzymatic reaction product, dephosphorylated LPS, is a non-toxic substance for mammals, and acts as a partial antagonist on the LPS receptor complex. Unlike other potential treatments, AP has been shown to act at the front end of the inflammatory cascade. By doing so, it may eliminate the root cause of the SIRS, and prevent the progression into sepsis and septic shock. Also, in ongoing sepsis, it cuts out the peaks of inflammatory responses induced by new LPS, which enters the systemic circulation after a septic insult. In addition, AP acts in the dephosphorylation of extracellular ATP via adenosine diphosphate (ADP) and adenosine monophosphate (AMP) into adenosine (Kiffer-Moreira et al 2014), with ATP having pro-inflammatory effects, whilst adenosine exhibits anti-inflammatory and tissue-protective effects (Day et al 2006).

Two clinical studies previously performed with purified bovine intestinal AP (BiAP) generated clinical and biomarker data supporting the use of AP as a therapeutic intervention to improve the outcome of AKI associated with sepsis (Heemskerk et al 2009; Pickkers et al 2012). In the latter APREN-01-01 study, a prospective, randomized clinical study, BiAP significantly improved kidney function (determined by a composite endpoint of

creatinine clearance, requirement for RRT, and duration of RRT) and significantly reduced the length of ICU stay. In addition, the duration of mechanical ventilation tended to be reduced, although not significantly. Moreover, a range of markers of systemic inflammation, renal function, and renal damage in blood and urine demonstrated significant improvement, suggesting that a systemic anti-inflammatory effect induced by the treatment resulted in fast recovery and prevention of further kidney damage. In these relatively small studies (n = 36, each) no significant effects on mortality could be observed.

Previous studies with BiAP in non-patient volunteers and in patients with sepsis and end-organ failure have established the tolerability and potential efficacy of BiAP. Following these encouraging positive results, AM-Pharma B.V. has developed a recombinant human chimeric form of AP, from parts of the intestinal and the placental isoforms (details are provided in the investigator's brochure [AM-Pharma B.V. 2014]).

Recombinant human AP is encoded by a human intestinal AP sequence, wherein the sequence encoding the crown domain has been substituted with the corresponding human placental AP sequence. Recombinant human AP is produced in Chinese hamster ovary cells using state-of-the-art mammalian cell culture technology. The investigational product is a concentrate for solution for infusion-containing recAP as active ingredient. AM-Pharma B.V. has developed recAP as a medicinal product to be used via intravenous (IV) infusion for the treatment of SA-AKI. In line with pre-clinical and clinical studies using purified BiAP, pre-clinical studies with recAP indicated that it asserts potent anti-inflammatory activity preserving function and histological integrity of the affected kidneys. In rodent and porcine models of AKI induced by ischemia-reperfusion injury or systemic endotoxemia, a single-dose administration of recAP consistently inhibited markers of inflammation and reduced kidney damage and functional impairment as determined by serum creatinine, blood urea nitrogen (BUN), creatinine clearance, and histopathological examination (AM-Pharma B.V. 2014). Its therapeutic activity is thought to be exerted through dephosphorylation and detoxification of pro-inflammatory substrates such as LPS, ATP and ADP. Adenosine triphosphate and ADP are highly potent triggers of inflammation released in damaged kidney tissue. In vitro, recAP inhibits immunological activation and chemotaxis of freshly isolated human leukocytes as well as platelet activation and aggregation induced by ATP and ADP. Therapeutic application of recAP is predicted to have potent anti-inflammatory and tissue-protective activity in patients suffering from AKI.

2 Study 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 of the study are as follows:

- To investigate the safety and tolerability of recAP in patients with SA-AKI.
- To investigate the pharmacokinetics (PK) of recAP in a subset of patients with SA-AKI (in the first 120 patients from Part 1 only).
- 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 that 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:
    - Kidney function markers (e.g., urine and serum creatinine, BUN/urea clearance, sodium, proteinuria, fractional excretion of sodium and urea, and urine output])
    - Tubular injury biomarkers (e.g., kidney injury molecule-1 [KIM-1], interleukin 18 [IL-18], alpha-glutathione s-transferase [GST-alpha])
    - Biomarkers for systemic inflammation (e.g., IL-6, C-reactive protein [CRP], lipopolysaccharide-binding protein [LBP])
    - Glomerular filtration rate (eGFR by chronic kidney disease epidemiology collaboration [CKD-EPI])
    - APACHE II score (<25, ≥25)

- Timing from first diagnosis of SA-AKI to start of recAP treatment, e.g., in time intervals (0 to < 6 hours, 6 to < 12 hours, 12 to < 18 hours, 18 to 24 hours).
- Baseline AKIN stage (stage 1, stage 2, stage 3)

Additional parameters may also be considered (e.g., urine output [over or under 200 mL/day], diabetes mellitus [yes or no], hypertension [yes or no], age [< 55 years old, ≥ 55 years old and < 70 years old, or ≥ 70 years old], sex (male or female), risk scores [high or low]).

3 Investigational Plan

3.1 Study Design

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

Approximately 100 sites will participate in the study; approximately 75 in the European Union and approximately 25 in the United States and Canada. Participating sites will be required to adhere to the Surviving Sepsis Campaign 2012 (Delinger et al 2013) and KDIGO® (Kidney Disease: Improving Global Outcomes) Clinical Practice Guideline for AKI recommendations (KDIGO Acute Kidney Working Group 2012).

A minimum of 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 continued 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 with an estimated 50 patients during the interim analysis. Patients enrolled during Part 1 and interim analysis will be randomly assigned to receive, by 1-hour IV infusion, either placebo (Part 1; n1 = 30) or one of 3 different doses of recAP (Part 1; 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 by 1-hour IV infusion once daily for 3 days (Days 1, 2, and 3). The interim analysis on the primary endpoint will be performed on all the data collected after the 120th patient of Part 1 has completed the Day 7 visit of the study to select the dose to be administered in Part 2. The dose chosen will be the optimal dose of recAP on the primary endpoint in Part 1, provided there are no safety issues with that dose as judged by the data monitoring committee (DMC). In Part 2, patients will be randomly assigned to receive, by 1-

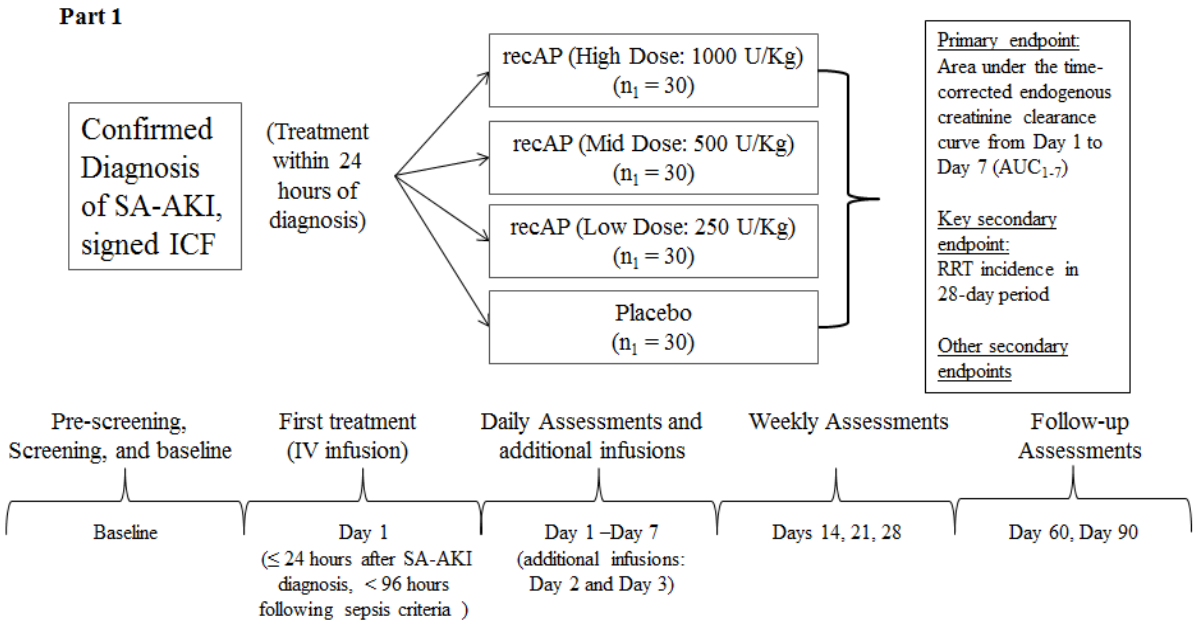
hour IV infusion, either placebo (n2 = 85) or the dose of recAP (n2 = 85) selected during the interim analysis. Patients recruited during the interim analysis period to the dose selected in Part 2 will form part of the Part 2 populations, but those recruited to the doses that are not selected will be included in the Part 1 population.

Each part involves the following schedule of events: potential patients who have been admitted to the ICU or intermediate care unit will undergo a pre-screening to assess the presence of SA-AKI, will provide informed consent, and will undergo screening assessments to determine eligibility. As soon as possible when all inclusion and none of the exclusion criteria are met (see Section 4.1), and after confirmation of continuing (i.e., not resolving) AKI by a fluid-corrected serum creatinine assessment (see Section 13.3) 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 first diagnosis of sepsis.

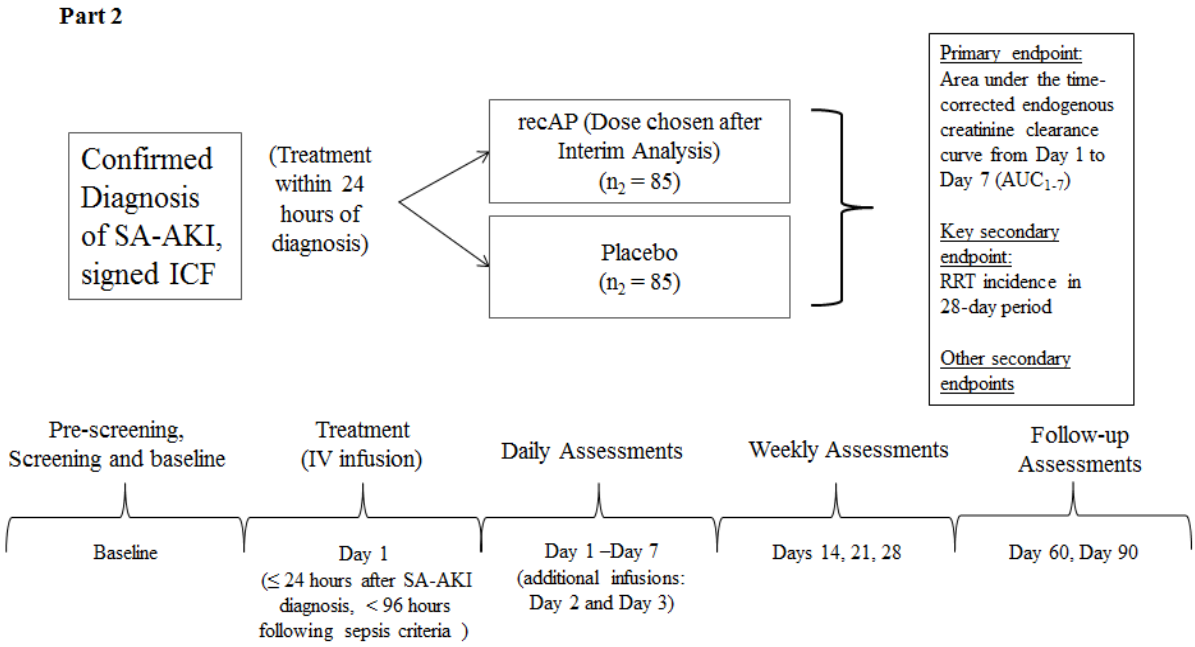
Assessments will be performed during a total of 12 visits, at the following intervals: daily from Day 1 to Day 7 ± 1 day (7 visits); weekly on Day 14 ± 2 days, Day 21 ± 3 days, and Day 28 ± 3 days (3 visits); and follow-up assessments will be completed on Day 60 ± 5 days and Day 90 ± 10 days (2 visits). The Schedule of Assessments is presented in Section 13.1 (Table 13-1) and the Flowchart of Assessments is presented in Section 13.2 (Figure 13-1).

Due to potential unblinding, it is not allowed to locally measure AP activity in the blood up to Day 14 (inclusive), irrespective of whether the patient is in the ICU or intermediate care unit or in the ward.

Figure 3–1 Study Design



Interim analysis to determine optimal active dose to compare with placebo in Part 2



Abbreviations: ICF = informed consent form; IV = intravenous; recAP = recombinant human alkaline phosphatase; RRT = renal replacement therapy; SA-AKI = sepsis-associated acute kidney injury.

3.1.1 Rationale of Study Design

As there are no guidelines for the development of drugs for the indication SA-AKI, the proposed design 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 US regulatory agencies.

The dosing schedule is described in the dosing rationale section. To determine the optimal dose the study has been set up with an adaptive study design including 2 parts, with dose selection based on an interim analysis after the 120th patient in Part 1 has completed the Day 7 visit. The 3 active doses in Part 1 are selected based on a combination of information from previous clinical studies conducted with BiAP, and pre-clinical animal models and PK modeling and simulation in a Phase 1 healthy volunteer study with recAP. Assuming comparable safety profiles, the optimal dose will be selected at the interim analysis by an independent DMC. The primary endpoint, area under the time-corrected endogenous creatinine clearance curve, 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 the first 120 patients from Part 1 only, 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).

3.1.2 Dose Rationale

No dose-limiting toxicity was observed in pre-clinical and clinical studies with recAP up to daily doses of 16 mg/kg (10 000 U/kg) and 32 mg/kg (20 000 U/kg) maintained for 14 days in Göttingen mini-pigs, and in healthy volunteers for a single dose up to 3.2 mg/kg (2000 U/kg) and for multiple doses up to 3 daily doses of 1.6 mg/kg (1000 U/kg). Therefore, the dose rationale is primarily based on: 1) observed Phase 1 recAP PK parameters; 2) trough

levels (293 U/L, corresponding to 0.171 mg/L recAP) obtained in the APREN-01-01 study; and 3) exposure maintained during the APREN-01-01 study and associated with clinical activity. The 3 dose level schedules proposed for the first part of this Phase 2 study, in addition to the placebo group, are shown in Table 3–1.

Table 3–1 Proposed Dose Groups for AP-recAP-AKI-02-01 Protocol

Dose group	$AUC_{0\text{-}inf}$ (h•U/L)	Fold (over BiAP)	C_{min} (U/L)	Fold (over BiAP)	t_{trough} (h)
250 U/kg or 0.40 mg/kg qd ×3	45 762 (26 225 - 75 202)	2.29	C_{min1}: 120 (74 - 187) C_{min2}: 183 (113 - 287)	0.41 0.62	$t_{trough1}$: 7.2 $t_{trough2}$: 35.0 $t_{trough3}$: 63.2
500 U/kg or 0.80 mg/kg qd ×3	90 901 (50 782 - 156 914)	4.55	C_{min1}: 241 (140 - 391) C_{min2}: 369 (213 - 599)	0.82 1.26	$t_{trough1}$: 18.7 $t_{trough2}$: NR $t_{trough3}$: 92.1
1000 U/kg or 1.60 mg/kg qd ×3	184 347 (102 688 - 301 544)	9.22	C_{min1}: 486 (288 - 742) C_{min2}: 746 (443 - 1136)	1.66 2.54	$t_{trough1}$: NR $t_{trough2}$: NR $t_{trough3}$: 144
BiAP (APREN-01-01) 67.5 U/kg (10 min) + 132.5 U/kg/24 h for 48 h	20 000 (estimated)	NA	293 ± 257 ('trough')	NA	NA

Abbreviations: BiAP = bovine intestinal alkaline phosphatase; Cmin = observed minimum plasma or serum concentration after administration; h = hour; min = minute; NA = not available; NR = not recorded; qd = every day (quaque die).
C_{min} is determined just before re-infusion (C_{min1} at t = 24 h; C_{min2} at t = 48 h).
Trough is set at 293 U/L (based on APREN-01-01 study).
T_{trough} reflects the time to reach trough ($t_{trough1}$ after first infusion, $t_{trough2}$, etc).

Using PK simulation experiments, these schedules achieve the area under the concentration-time curve from zero up to an infinite time ($AUC_{0\text{-}inf}$) and observed minimum plasma or serum concentration after administration (C_{min}) levels that are similar to or exceed the presumed minimal requirements based on BiAP studies APSEP-02-01 and APREN-01-01. Furthermore, these dose groups are likely to achieve significantly different exposures when comparing 3 groups of 30 patients (as informed by the observed PK variability in Phase 1 patients). Analyzing PK simulations, compared to the APREN-01-01 study results the recAP low-dose group is expected to achieve sufficient exposure with C_{min} dropping below target trough before re-infusion, whereas the mid-dose and high-dose groups exceed target exposure and C_{min}. If clinical activity is observed to be different in Phase 2 at interim

analysis, the proposed set up of dose groups is likely to enable a clear decision on optimal recAP dose for continuation into the second part of the study.

3.1.3 Individual Benefit/Risk Considerations

Benefits for patients with sepsis and AKI are anticipated based on pharmacodynamic (PD) data and data from 2 Phase 2 studies described in the investigator's brochure. In the APSEP 02-01 study, BiAP was associated with a non-significant survival benefit versus placebo and clinically important benefits on renal function parameters of serum creatinine, inflammatory and kidney damage biomarkers and dialysis requirements. In the APREN01-01 study, BiAP significantly improved kidney function (determined by a composite endpoint of creatinine clearance, requirement for RRT and duration of RRT) and significantly reduced the length of ICU stay. There was a non-significant trend toward a reduction in the requirement for mechanical ventilation and a significant improvement in biomarkers of systemic inflammation, of renal function, and of renal damage in blood and urine, suggesting that a systemic anti-inflammatory effect induced by the treatment resulted in fast recovery and prevention of further kidney damage.

No safety concerns were identified in either of these Phase 2 clinical studies with BiAP or in the Phase 1 study in 51 healthy volunteers with recAP.

4 Patient Selection and Withdrawal Criteria

4.1 Selection of Study Population

A minimum of 290 patients will be enrolled (Part 1: at least 120 patients;
Part 2: 170 patients) at approximately 100 sites (75 sites in the European Union and 25 sites in the United States and Canada). Patients will be enrolled and randomly assigned to study drug only if all of the inclusion criteria and none of the exclusion criteria are met. Recruitment will continue and additional patients may be recruited during the interim analysis (approximately 50 patients).

Deviations from the inclusion and exclusion criteria are not allowed because they can potentially jeopardize the scientific integrity of the study, regulatory acceptability, and, most importantly, patient safety. Therefore, strict adherence to the criteria as specified in the protocol is essential.

4.1.1 Inclusion Criteria

Each patient must meet all of the following criteria to be enrolled in this study:

1. Has informed consent form (ICF) signed according to local rules and approved regulations.
2. Is aged 18 to 85 years, inclusive.
3. Is admitted to the ICU or intermediate care unit.
4. Has diagnosis of sepsis (<96 hours prior to first study drug administration or < 72 hours prior to AKI diagnosis), according to criteria defined by the American College of Chest Physicians/Society of Critical Care Medicine (Bone 1992]), based on:
    a. Has a proven or strongly suspected bacterial infection and
    b. Have at least 2 of the following 4 SIRS criteria within a timeframe of 72 hours at time of AKI diagnosis. Note: it is not required that symptoms are present simultaneously at study randomization:
        i. Has a core temperature > 38°C or < 36°C.
        ii. Has a heart rate > 90 beats/minute (unless the patient has a medical condition known to increase heart rate or is receiving treatment to prevent tachycardia).
        iii. Has a respiratory rate > 20 breaths/minute, $PaCO_2$ < 32 mm Hg or the use of mechanical ventilation for an acute respiratory process.
        iv. Has a white cell count > 12 000/mm^3 or < 4000/mm^3 or a differential count showing > 10% immature neutrophils (band cells).
5. Has first diagnosis of AKI, defined as any of the following:

AKI Stage 1 or greater, according to the following Acute Kidney Injury Network (AKIN) criteria (Note: adjusted in regards to time-window):

a. Increase (absolute) in serum creatinine > 26.2 µmol/L (0.30 mg/dL) compared with a serum creatinine value within the previous 48 hours, or presumed to have occurred in the previous 48 hours when compared to a reference* creatinine value

b. Increase (relative) in serum creatinine to > 150% (> 1.5–fold) compared with a serum creatinine value in the previous 48 hours or presumed to have occurred in the previous 48 hours when compared to a reference* creatinine value.

*The reference creatinine value is a serum creatinine value in 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

c. Urinary output < 0.5 mL/kg/h for > 6 hours following adequate fluid resuscitation when applicable, in the absence of underlying primary renal disease.

6. When the diagnosis of AKI is made according to one of the AKIN serum creatinine criteria (absolute or relative increase, see inclusion criteria 5a and 5b), continuing AKI needs to be confirmed by a confirmative serum creatinine measure (that is corrected for fluid administrations), defined as no decrease in serum creatinine ≥ 26.2 µmol/L (≥0.30 mg/dL). The result must be available prior to randomization, within 24 hours after the primary AKI diagnosis so that administration of the first study treatment can be started within 24 hours after AKI diagnosis.
7. When the AKI diagnosis is made according to the AKIN urine output criteria (urinary output < 0.5 mL/kg/h for > 6 hours, see inclusion criterion 5c), the oliguria or anuria should still meet the AKIN urine output criteria prior to randomization and study drug administration; administration of study treatment must be started within 24 hours after first AKI diagnosis.

4.1.2 Exclusion Criteria

Patients meeting any of the following criteria will be excluded from the study:

1. Woman of childbearing potential with a positive pregnancy test (blood or urine), pregnant, or breastfeeding.
2. Weighs more than 115 kg (253 lb).
3. Has life support limitations (i.e., do not intubate, do not dialyze, do not resuscitate).

4. Is known to be human immunodeficiency virus positive.
5. Has urosepsis.
6. Is already on dialysis (RRT) or a decision has been made to initiate RRT within 24 hours after planned start of study drug administration.
7. Is receiving immunosuppressant treatment or is on chronic high doses (high-dose therapy exceeding 2 weeks of treatment) of steroids equivalent to prednisone/prednisolone 0.5 mg/kg/day, including solid organ transplant patients. Patients with septic shock treated with hydrocortisone (e.g., 3 × 100 mg) can be included.
8. Is expected to have rapidly fatal outcome (within 24 hours).
9. Has known, confirmed fungal sepsis.
10. Has advanced chronic liver disease, confirmed by a Child-Pugh score of 10 to 15 (Class C).
11. Has acute pancreatitis with no established source of infection.
12. Has participated in another investigational study within 30 days prior to enrollment into the study.
13. Is not expected to survive for 28 days due to medical conditions other than SA-AKI, e.g., end-stage cancer, end-stage cardiac disease, cardiac arrest requiring cardiopulmonary resuscitation or with pulseless electrical activity or asystole within the past 30 days, end-stage lung disease, and end-stage liver disease.
14. Has known prior history of CKD with a documented sustained estimated GFR (eGFR) < 60 mL/min by a commonly used formula such as Modification of Diet in Renal Disease (MDRD) or Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI), a measured GFR < 60 mL/min, or a known history of persistent creatinine level equal or greater than 150 µmol/L (1.70 mg/dL) prior to entry for reasons other than the current sepsis condition.
15. Has diagnosis of malaria or other parasite infections.
16. Has burns on > 20% of body surface.
17. Has had AKI diagnosis according to the AKI inclusion criteria for a period longer than 24 hours prior to study drug administration.
18. Is anticipated to be treated with non-continuous RRT from Day 1 to Day 7.

19. During Day 1 to Day 7 continuous RRT is anticipated to be started or stopped **not** according to per protocol criteria (Section 6.3.9).
20. The AKI is most likely attributable to other causes than sepsis, such as nephrotoxic drugs (NSAIDs, contrast, aminoglycosides) and renal perfusion-related (acute abdominal aortic aneurysm, dissection, renal artery stenosis).
21. Improvement in serum creatinine of at least 0.30 mg/dL or (26.2 µmol/L) prior to administration of the study drug.
22. Patients who use nephrotoxic medication e.g., NSAIDs, angiotensin-converting enzyme inhibitors, gentamycin, tobramycin) and who fulfill the SA-AKI inclusion criteria at screening are not eligible if the use of this nephrotoxic medication is to continue when alternative, medically appropriate, non-nephrotoxic medication is available . (Note: this is according to KDIGO Clinical Practice Guideline for AKI recommendations [KDIGO Acute Kidney Working Group 2012]) to avoid nephrotoxic medication, where possible).
23. Has a history of known IV drug abuse.
24. Is an employee or family member of the investigator or study site personnel.
25. Has active hematological malignancy.

4.2 Withdrawal of Patients From the Study

The duration of the study for a patient is defined from the date that signed written informed consent is provided until the last follow-up visit on Day 90.

4.2.1 Reasons for Withdrawal/Discontinuation

Patients may withdraw from the study at any time and for any reason without prejudice to their future medical care by the investigator or at the study site. Investigators should attempt to determine the cause of withdrawal and, if agreed by the patient, to let the patient return for the Day 90 visit (last safety follow-up visit). The extent of a patient's withdrawal from the study (i.e., withdrawal from further study treatment, withdrawal from active participation in the study, withdrawal from any further contact) should be documented in the eCRF. Efforts should be made to follow-up the randomized and withdrawn patients as much as possible, i.e., to the extent that the patients agree to.

Treatment with study drug will be stopped for any of the following reasons:

1. Any clinical adverse event (AE), laboratory abnormality, or intercurrent illness which, in the opinion of the investigator, indicates that continued treatment with study drug is not in the best interest of the patient.
2. The patient withdraws consent or the investigator or sponsor decide to discontinue the patient's participation in the study.
3. AM-Pharma B.V. terminates the study

Additionally, treatment with study drug may be stopped in case of protocol non-compliance or violations. In this case, the investigator will contact the Medical Monitor to discuss risk/benefit balance of continued treatment with study drug.

In case study drug administration is discontinued prematurely the patient will preferably continue follow-up in the study as per protocol for safety and to allow for intent-to-treat (ITT) analysis. If study drug administration is interrupted for any reason, re-starting of study drug should be discussed with the Medical Monitor.

The reason for and date of study drug discontinuation and/or the reason for and date of withdrawal from the study must be recorded in the electronic case report form (eCRF). If study drug is discontinued because of an AE or a clinically significant abnormal laboratory test result, evaluations will continue until the event has resolved or stabilized or until a determination of a cause unrelated to the study drug or study procedure is made. The specific event or laboratory finding(s) must be documented.

4.2.2 Handling of Withdrawals

When a patient withdraws from the study, the investigator will record the reason(s) for withdrawal on the relevant page of the eCRF. All patients who withdraw from the study prematurely will preferably undergo end-of-study (Day 28) assessments immediately upon discontinuation. Investigators should attempt to determine the cause of withdrawal and, if agreed by the patient, to let the patient return for the Day 90 visit (last safety only follow-up visit).

It is highly desirable to obtain follow-up safety data on any patient withdrawn because of an AE or serious adverse event (SAE). In every case, efforts must be made to undertake

protocol-specified, safety, follow-up procedures (Section 6.3.14). All data from withdrawals will be included in the final ITT analyses.

Patients who fail to return for final assessments in general will be contacted by the site in an attempt to have them comply with the protocol; attempts to contact patients will be recorded in the eCRF. Three documented attempts to follow up of which at least one is a registered letter are considered a reasonable effort to obtain information before subject is considered lost to follow-up.

4.2.3 Replacements

As the sample size determination is based on the number of patients required for the ITT analysis, patients who are randomly assigned and subsequently withdraw prior to completion of the study will not be replaced.

5 Study Drugs

5.1 Method of Assigning Patients to Treatment Groups

Patients will be randomly assigned to receive either placebo or one of 3 doses of recAP using a 1:1:1:1 allocation ratio in Part 1. The randomization schedule will be stratified by site. Once it has been decided which is the 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 will generate a permuted block randomization schedule using SAS® software Version 9.2 or later (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 may not be assigned to another patient.

5.2 Treatments Administered

Study drug will be administered by 1-hour IV infusion as soon as possible on Day 1 after confirmation of continuing AKI, and on Days 2 and 3, 24 ± 1 hour after previous drug

administration, by trained staff in the ICU or intermediate care unit. A total of 50 mL (see Section 5.3) will be infused at a constant rate of 50 mL/hour, followed by a flushing of infusion lines with minimal 4 mL saline. 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.

Vital signs will be monitored during infusion on all treatment days (see Section 6.3.4).

Patients randomly assigned to receive recAP in Part 1 and during the interim analysis will receive one of the following 3 doses of recAP: 0.4 mg/kg (250 U/kg), 0.8 mg/kg (500 U/kg), or 1.6 mg/kg (1000 U/kg). Patients will receive study drug by 1-hour IV infusion once daily for 3 days (Days 1, 2, and 3). The exact volume of recAP or placebo prior to dilution will be determined according to the patient's weight. Patients weighing between 95 to 115 kg will receive the same dose as that for patients weighing 100 kg. A detailed medication preparation instruction sheet will be provided. A table with weight ranges and pre-calculated corresponding volumes is provided in Section 13.4. The maximum weight is limited to 115 kg (253 lb).

Treatment with recAP will not be repeated in the same patient beyond the scope of the protocol. All patients should be monitored closely for signs of adverse reactions. Due to the IV route of administration, local irritation and hematoma at the infusion site may occur (when administered through a peripheral line). No specific measures are required when extravasation occurs.

5.3 Identity of Investigational Product

Alkaline phosphatases are homodimeric enzymes responsible for dephosphorylation of monoesters of phosphoric acid by hydrolysis. Recombinant human AP is a full-length human chimeric AP derived by recombinant technology and produced in Chinese hamster ovary cells. Recombinant human AP has a projected mass of approximately 105 kDa based on the amino acid sequence derived from the DNA sequence and approximately 130 kDa as a fully glycosylated molecule.

Recombinant human AP is supplied as an IV solution for infusion with an activity of 5000 U/mL (units of AP activity) and a content of 40 mg per vial at 8.0 mg/mL (protein

concentration) in an aqueous buffer containing 20 mM citrate, 250 mM sorbitol, 2 mM $MgCl_2$, 50 µM $ZnCl_2$, and with a pH of 7.0.

Matching placebo for use in controlled studies consists of an IV solution for infusion containing an aqueous buffer containing 20 mM citrate, 250 mM sorbitol, 2 mM $MgCl_2$, 50 µM $ZnCl_2$, and with a pH of 7.0.

Recombinant human AP is supplied as a clear, colorless, pyrogen-free solution in 10-mL type 1 glass vials with a Teflon-coated bromobutyl rubber stopper and "Flip-Tear Up" overcap. 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 with a Teflon-coated bromobutyl rubber stopper and "Flip-Tear Up" overcap. Each vial contains 5 mL of placebo solution.

Patient doses will be prepared from 4 vials in a combination of recAP drug product and placebo. All content of all 4 vials will be used to fill an IV dosing syringe (50 mL). According to Table 13-2, part of the medication volume might be discarded in order to obtain an appropriate volume corresponding to the body weight of the patient, followed by addition of physiological saline solution to a total volume of 50 mL.

Detailed instructions are provided in the study drug preparation manual.

All study drug is to be stored in a lockable storage facility under appropriate and monitored pharmacy conditions. Alkaline phosphatase vials are to be stored at 2°C to 8°C and protected from light and moisture until preparation for use.

Study drug needs to be prepared within 3 hours before dosing. Prior to use, the medication will be prepared according to the pharmacy manual; reconstituted medication can be stored at 2 to 8°C for up to 3 hours and/or a maximum of 1 hour at room temperature. If applicable, the study drug will be re-labelled (as per current regulations) by the hospital pharmacy. Additional details will be provided in the study drug preparation manual.

The hospital/study pharmacist is responsible for receipt of the study drug from PPD and for transfer to the ICU or intermediate care unit.

5.4 Management of Clinical Supplies

5.4.1 Study Drug Packaging and Storage

Recombinant human AP active 8 mg/mL and matching placebo will be prepared in randomized treatment packs comprising 3 daily doses, each of 4 vials (in any combination of recAP drug product and placebo) and shipped by PPD to the clinical site. Each pack will contain a (randomized) dosage for 1 patient and will contain a sufficient quantity for dispensing during the 3-day double-blind treatment period.

The labeling of recAP and matching placebo will be in accordance with applicable local requirements in the countries where this study is conducted.

Recombinant human AP must be stored in a secure area protected from light and kept at a controlled temperature of 2°C to 8°C.

Full details of study drug packaging, storage, and shipment will be provided in the pharmacy manual.

5.4.2 Test Article Accountability

The investigator has the responsibility to maintain accurate records of receipt of all test articles, including dates of receipt. In addition, accurate records will be kept regarding when and how much test article is dispensed and used by each patient in the study. Reasons for deviating from the expected dispensing regimen as instructed must also be recorded. At the completion of the study, to satisfy regulatory requirements regarding drug accountability, all study drugs will be reconciled and retained or destroyed according to applicable regulations.

The drugs supplied for this study are only intended for use by patients in this study. A drug accountability record must be maintained by the persons responsible for preparing and administering the study drug at the ICU or intermediate care unit. The drug accountability record will be provided and will be collected by the monitor at the end of the study. The monitor will also arrange for the secure disposal of unused and returned medication at the end of the study.

5.5 Management of Medication Errors or Overdose

5.5.1 Medication Errors

Since the study drug will be prepared, double-checked, and administered by trained hospital staff, and since the dose levels under study have not caused any SAEs in healthy volunteers, the risks for potential hazardous or life-threatening events due to medication errors is considered to be minimal. Hospital staff will prepare the study drug by adjusting downward the maximum volume of 20 mL based on body weight according to the table provided in the medication preparation instruction sheet (see Section 5.2). Study site staff will have checks in place to verify the preparation of the medication solution. Discarded volume will be collected in a vial for review of medication administration by the monitor during a regular monitoring visit.

No specific treatment is available for medication errors and it is unknown whether or not specific symptoms will occur. If a medication error occurs, as a general rule supportive care and treatment of symptoms should be provided.

5.5.2 Treatment of Overdose

In healthy volunteers the maximum administered single dose that did not result in any SAEs was 3.2 mg/kg (2000 U/kg). The maximum multiple dose that was administered to healthy volunteers was 1.6 mg/kg (1000 U/kg) per day for 3 days without any SAEs. As an example of a possible dose error (e.g., administration of the highest study dose [i.e., 1.6 mg/kg {1000 U/kg} which is the dose for a 100-kg person] to a 50-kg person), 1-day administration of study drug still would not exceed the tested maximum dose in healthy volunteers.

No specific treatment is available for overdose and it is unknown whether or not specific symptoms will occur. If an overdose occurs, as a general rule supportive care and treatment of symptoms should be provided.

5.6 Blinding

All persons involved in the study (including but not limited to the patient, site staff, AM-Pharma B.V. team members, and PPD team members) 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 (see Section 7.7.6).

The determination of AP activity, often part of the routine clinical chemistry panel, could lead to unblinding and to erroneous interpretation of liver function, as the recAP administered will increase the AP activity, exceeding many times the reference range AP levels. For that reason, AP activity levels from samples taken during the first 14 days of the study are not allowed to be reported to the study team members or to any other study staff member involved in the conduct of the study. After Day 14, considering the terminal half-life of recAP, AP activity levels are expected to have returned to normal levels. Therefore, results of AP activity in samples that are taken after Day 14 may be reported.

5.6.1 Breaking the Blind

A patient's treatment assignment will not be broken until the end of the study unless knowledge of the treatment assignment the patient received is necessary to guide medical treatment of the patient. In the event that the blind needs to be broken because of a medical emergency, the investigator may unblind an individual patient's treatment allocation. The investigator should contact the Medical Monitor to discuss the medical emergency and the reason for revealing the actual treatment received by that patient unless immediate unblinding is necessary to ensure the patient's safety. The treatment assignment will be unblinded through IVRS. Reasons for treatment unblinding must be clearly explained and justified in the eCRF. The date on which the code was broken together with the identity of the person responsible must also be documented.

5.7 Concomitant Therapy

Concomitant medication use, including the drug name (brand or generic), dose, and dates of administration, will be recorded in the eCRF beginning on baseline to Day 28. Changes in dosage of any concomitant medication will be recorded in the eCRF, except for IV medications that undergo frequent adjustments during the same day or over time, such as insulin, vasopressors, inotropics, diuretics, sedatives, metoprolol, and potassium. In these cases, only estimated mean daily dose or mean daily dose as calculated by the system used will be recorded. Concomitant medications will include all prescription drugs, including vitamins and minerals, herbal products, and over-the-counter medications. Only brand names

of nutritional products are to be recorded, while the single constituents of nutritional products need not be recorded.

Administered resuscitation fluids should only be recorded as total daily volume in the concomitant medication section in the eCRF. Blood products should be recorded separately.

5.7.1 Concomitant Medications to be Avoided

A list of nephrotoxic concomitant medications that should be avoided, if possible, is provided in the study procedures manual (SPM). Based on findings that emerge during study conduct, the SPM will be updated as appropriate to include any additional concomitant medications that should be avoided, if possible.

After randomization until the resolution of the current AKI episode, the administration of nephrotoxic drugs such as contrast agents, NSAIDs, angiotensin-converting enzyme inhibitors, gentamycin, or tobramycin should be avoided where possible, as recommended in the KDIGO Clinical Practice Guideline for AKI recommendations (KDIGO Acute Kidney Working Group 2012).

6 Days, Assessments, and Procedures

The Schedule of Assessments is presented in Section 13.1 (Table 13-1) and the Flowchart of Assessments is presented in Section 13.2 (Figure 13-1).

Visits Day 1 through Day 7 are calculated on a 24-hour clock starting from the start of the first study drug administration. One visit day might cover 2 calendar days. After the Day 7 visit, visit days are similar to calendar days. Counting is to start on the calendar day of the first study drug administration.

6.1 Days

6.1.1 Pre-screening, Screening and Baseline

Fully informed consent will be obtained before any study-specific procedures are performed. Country-specific regulations regarding the collection of informed consent and personal data will be followed at each site. It is anticipated by the very nature of the study that many patients who will be eligible for this protocol due to their severe underlying condition will not be able to provide informed consent themselves for various reasons including sedation or

unconscious state. In a situation where a patient is unable to provide consent, the patient's legally authorized representative may provide written consent, as approved by the institutional-specific guidelines. Informed consent may be obtained from an independent consulting physician or obtained on the basis of emergency study protocol by the investigator in countries where regulation and institution guidelines permit and where regulatory authorities and/or institutional review board (IRB)/independent ethics committee (IEC) have approved the procedure for the study. This form of consent can only be used when the patient and legally authorized representative are unable to provide consent. In cases where the initial informed consent is obtained from a legal representative, an independent consulting physician, or by investigator, the patient must also give written informed consent as soon as they are able.

Pre-identified patients (or their legal representative, or independent physician, or other according to local regulatory requirements) who meet all of the inclusion criteria and none of the exclusion criteria that can be assessed in a non-invasive manner will be asked if they are willing to participate in the study by the attending physician. If they agree, patients (or their legal representative, or independent physician, or other according to local regulatory requirements) will be informed verbally about the study schedule and potential risks. They will also receive written information on the study. They will be given adequate time to read the information and sufficient opportunity to ask any questions. A patient should not enter the study if he/she (or his/her legal representative) has not understood the written and verbal information provided and/or if there is no signed and dated ICF. Patients (or their legally authorized representative) will have the opportunity to have any questions answered before signing the ICF. The person obtaining consent must address all questions raised by the patient or their legally authorized representative. The person obtaining consent will also sign and date the ICF. A copy of the written information and the signed ICF will be provided to the participant (or his/her legal representative), whereas the original version will be retained by the investigator. It is the responsibility of the investigator to ensure that the patient meets the study enrollment criteria.

After informed consent has been obtained, each participant will be assigned a unique patient study identification number, consisting of 6 digits, 3 digits reflecting the site number followed by 3 digits reflecting the patient number. An IVRS supplies a patient number at screening and retains a unique randomization number for all patients. Once the screening assessments are performed (including assessment of all inclusion and exclusion criteria), data

have been reviewed by an investigator, and the patient is considered to be eligible (including the confirmatory fluid-corrected serum creatinine [see Section 13.3]), the investigator will contact the IVRS to randomly assign the patient a treatment number through IVRS. Reasons for screen failure of patients with an allocated patient number will be recorded in the IVRS system. The assessments performed before randomization during screening, and after randomization at baseline and on Day 1 are listed in the Schedule of Assessments presented in Section 13.1 (Table 13-1) and the Flowchart of Assessments is presented in Section 13.2 (Figure 13-1).

6.1.2 Day 1 to Day 3: Treatment Days

Study drug will be administered on Day 1, as soon as possible when criteria are met, immediately after confirmation of continuing (i.e., not resolving) AKI either by a fluid-corrected serum creatinine assessment (see Section 13.3) or by assessment of continuation of decreased urine output. Treatment must be administered as soon as possible and within 24 hours, at the latest, after first meeting the criteria of SA-AKI.

Study drug will also be administered on Days 2 and 3 at 24 ± 1 hour after the previous administration. Exact administration clock times will be recorded.

The assessments performed from Day 1 to Day 3 are listed in Sections 6.2.3 to 6.2.5 and in the Schedule of Assessments presented in Section 13.1 (Table 13-1) and the Flowchart of Assessments is presented in Section 13.2 (Figure 13-1).

6.1.3 Day 4 to Day 28: Assessments and Procedures

Assessments and procedures to be performed from Day 4 to Day 28 are listed in Sections 6.2.3 to 6.2.5 and in the Schedule of Assessments presented in Section 13.1 (Table 13-1) and the Flowchart of Assessments is presented in Section 13.2 (Figure 13-1). Day 28 is considered end of study.

6.1.4 Days 60 and 90: Follow-up Assessments and Procedures

Assessments and procedures to be performed at follow-up are listed in Sections 6.2.3 to 6.2.5 and in the Schedule of Assessments presented in Section 13.1 (Table 13-1) and the Flowchart of Assessments is presented in Section 13.2 (Figure 13-1).

6.2 Study Assessments

6.2.1 Screening Assessments

Patients with AKI and a suspected or proven bacterial-induced sepsis might be considered to be enrolled in the study.

The following assessments and procedures will be performed during screening:

- AKI diagnosis and record site of infection and pathogen, if known
- Inclusion and exclusion criteria
- Signature of the ICF by the patient, or his/her legal representative, or independent physician, or other according to local regulatory requirements
- In case of liver disease, Child-Pugh score to exclude chronic liver disease (score of 10 to 15 [Class C])
- Pregnancy test (urine or blood) performed by local laboratory
- Collection of recent microbial test results, if available
- Collection of demographics and medical history
- Collection of recent hematology and clinical chemistry results, if available
- Verification that no concomitant medications that should be avoided are taken
- Shortly prior to randomization and study drug administration, a confirmatory fluid-corrected serum creatinine sample or confirmatory assessment of continuation of decreased urine output (see Section 13.3)
- When the diagnosis of AKI is made according to one of the AKIN serum creatinine criteria (absolute or relative increase, see inclusion criteria 5a and 5b), patients will be eligible for the study and can be randomly assigned when the fluid-corrected confirmatory serum creatinine sample, confirms the continuation of AKI according to the AKIN criteria for serum creatinine defined as no decrease in serum creatinine ≥ 26.2 µmol/L (≥ 0.3 mg/dL). When the AKI diagnosis is made according to the AKIN urine output criteria (urinary output < 0.5 mL/kg/h for > 6 hours, see inclusion criterion 5c), the oliguria or anuria should still meet the minimum AKIN urine output criterion prior to randomization and study drug administration.

6.2.2 Baseline Assessments

The following assessments will be performed at baseline after randomization (i.e., at study inclusion, not necessarily at ICU or intermediate care unit admission date):

- Vital signs (temperature, heart rate, respiratory rate, oxygen saturation, and blood pressure)
- Physical examination
- 12-lead electrocardiogram (ECG)
- Acute Physiology And Chronic Health Evaluation II (APACHE II) score
- Simplified Acute Physiology Score 2 (SAPS-2) score
- Sequential Organ Failure Assessment (SOFA) score
- Quality of life questionnaire (EQ-5D)
- Urine collection during 6 ± 1 hour period with registration of exact time of collection and determination of urine volume (only required when possible within the 24-hour time window from first AKI diagnosis to treatment)
- Local laboratory assessments
    - Hematology
    - Clinical chemistry, excluding AP activity
    - Note: Platelets, bilirubin, creatinine, and urine output are required to obtain a SOFA score.
- Central laboratory
    - Tubular injury biomarkers (e.g., KIM-1, IL-18, GST-alpha) in urine
    - Biomarkers for systemic inflammation (e.g., IL-6, CRP, LBP) in plasma/serum
    - Kidney function markers (e.g., urine and serum creatinine, BUN/urea clearance, sodium, proteinuria, fractional excretion of sodium and urea, and urine output)
    - Serology (immunoglobulin G [IgG], IgE, and total immunoglobulin) in plasma/serum
    - Anti-drug antibodies in plasma/serum

- AP activity (pre-dose) in plasma/serum

- For patients who are ventilated:
  - Non-mechanical ventilation: non-rebreathing mask, or nasal oxygen delivery (liters of oxygen/min, estimated daily average)
  - Mechanical ventilation (non-invasive and invasive):
    - Arterial partial oxygen pressure (PaO_2) (estimated daily average)
    - Fraction of inspired oxygen (FiO_2) and positive end-expiratory pressure (PEEP) (estimated daily average)
    - Tidal volume (estimated daily average) for invasive mechanical ventilation only, and
    - To enable calculation of P/F ratio: PaO_2 and FiO_2 must be captured at the same time point. Only one simultaneous measurement will be used. In case of multiple measurements of this fraction the worst value must be used.

- Concomitant medication

- Recording start and stop date, name, and mean daily dose of vasopressor and inotropic therapy for determination of shock-free days

- Time from first diagnosis of SA-AKI to start of recAP treatment

All screening and baseline assessments have to be performed within 24 hours from the first diagnosis of SA-AKI to first infusion of study drug.

6.2.3 Efficacy Assessments

The following assessments will be considered as efficacy measurements.

- Urine volume, urinary creatinine, and the average of two serum creatinine measurements will be used for the calculation of time-corrected endogenous creatinine clearance at all visits from baseline to Day 7, and will be calculated by the central laboratory. The measurements from Day 1 to Day 7, inclusive, will be used to calculate the primary endpoint. Only if reliable urine collection is possible, urine will be collected on Days 14, 21, and 28 for time-corrected endogenous creatinine clearance calculation (i.e., patient is in the ICU or intermediate care unit or hospital). If reliable urine collection is not possible, one serum creatinine sample still needs to be taken for calculating eGFR.

- Urine volume collection in a 6 ± 1 hour collection period at baseline and at all visits from Day 1 to Day 28, but only required if reliable urine collection is possible (i.e., patient is in the ICU or intermediate care unit or hospital)
- BUN clearance at all visits from Day 1 to Day 7.
- Urinary urea at baseline and at all visits from Day 1 to Day 28, only if reliable urine collection is possible (i.e., patient is in the ICU or intermediate care unit or hospital)
- Fractional excretion of sodium at all visits from baseline to Day 7
- Fractional excretion of urea at all visits from baseline to Day 7
- Proteinuria at baseline and at all visits from Day 1 to Day 90
- Tubular injury biomarkers, KIM-1, IL-18 and GST-alpha, in urine at baseline and at all visits from Day 1 to Day 28
- Serum creatinine at baseline and at all visits from Day 1 to Day 90
- Serum BUN at baseline and at all visits from Day 1 to Day 28.
- Systemic inflammatory serum biomarkers, e.g., IL-6, CRP, LBP, at baseline and at all visits from Day 1 to Day 28
- RRT incidence, considered as the key secondary endpoint, during the study period Day 1 to Day 28
- RRT duration, during the study period Day 1 to Day 28
- Incidence of dialysis dependency on Day 60 and Day 90
- SOFA score results at baseline, at all visits from Day 1 to Day 28 (when patient is in the ICU or intermediate care unit), and on the day of ICU or intermediate care unit discharge if discharged on a non-visit day. Note that platelets, bilirubin, creatinine and urine output are required to obtain a SOFA score.
- Quality of life, EQ-5D questionnaire at baseline, at ICU or intermediate care unit discharge, and Day 90
- Recording start and stop date, name, and mean daily dose of vasopressor and inotropic therapy for determination of shock-free days, at baseline and at all visits from Day 1 to Day 28, only required when patient is in the ICU or intermediate care unit

- Length of hospital and ICU or intermediate care unit stay by recording 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)

6.2.4 Safety Assessments

The following assessments will be considered safety measurements:

- Vital signs, including blood pressure, heart rate, oxygen saturation, respiratory rate, and body temperature at baseline and at all visits from Day 1 to Day 28. During the 3 dosing days (Day 1 to Day 3), repeated vital sign measurements will be performed (see Section 6.3.4).
- Physical examination at baseline and at all visits from Day 1 to Day 28
- 12-lead ECG at baseline and at visits on Days 3 and 14
- Local laboratory at baseline and at visits on Days 1, 3, 5, 7, 14, 21, and 28:
    - Hematology
    - Clinical chemistry, including liver function parameters (aspartate aminotransferase [AST], alanine aminotransferase [ALT], gamma-glutamyl transpeptidase [GGT] lactate dehydrogenase [LDH], and bilirubin; excluding AP)
- Serology (IgG, IgE and total immunoglobulin) at baseline and at visits on Days 14 and 28
- Anti-drug antibodies at baseline and at visits on Days 14, 28, 60, and 90. Samples taken on Days 60 and 90 will only be analyzed in case of a positive result on Day 28
- Concomitant medication at screening, baseline, and all visits from Day 1 to Day 28
- AEs during the duration of the study (see Section 6.3.14)
- Mortality (monitored continuously throughout the study)

6.2.5 Exploratory Assessments

The following assessments will be considered as exploratory measurements:

- PK analyses performed at all visits from Day 1 to Day 7 (inclusive) in the first 120 patients from Part 1 only
- Lung function. The following must be recorded during the study:
  - Ventilation modality (e.g., non-rebreathing mask, non-invasive mechanical ventilation, invasive mechanical ventilation) and start and stop dates
  - Non-mechanical ventilation: non-rebreathing mask, or nasal oxygen delivery (liters of oxygen/min, estimated daily average)
  - Mechanical ventilation (non-invasive and invasive):
    - Arterial PaO_2 (estimated daily average)
    - FiO_2 and PEEP (estimated daily average)
    - Tidal volume (estimated daily average) for invasive mechanical ventilation only
    - To enable calculation of P/F ratio: PaO_2 and FiO_2 must be captured at the same time point. Only one simultaneous measurement will be used. In case of multiple measurements of this fraction the worst value must be used.

6.3 Study Procedures

The investigator or designee will determine and document in a timely manner if abnormal outcomes (e.g., abnormalities on physical examination, vital signs, or laboratory parameters) are clinically significant. Details of AE reporting are provided in Section 6.3.14.3.

6.3.1 Demographics and Medical History

Demographics and medical history should be recorded at baseline (see Section 13.1 [Table 13-1] and Section 13.2 [Figure 13-1]). Height will be collected at baseline for calculation of body mass index (BMI; body weight (kg) / [height (m)]^2).

Medical history should be recorded with special emphasis for pre-existent renal disease and the use of immunosuppressant medication, steroids, and nephrotoxic drugs. The medical

history must be documented in the patient's chart and also recorded on the appropriate page of the eCRF.

6.3.2 Site of Infection and Pathogen

The location and identification of the pathogen causing the septic condition of the patient will be recorded at screening, if available (see Section 13.1 [Table 13-1] and Section 13.2 [Figure 13-1]).

6.3.3 Physical Examination

A complete physical examination will be performed at baseline and at all subsequent visits up to the Day 28 visit (see Section 13.1 [Table 13-1] and Section 13.2 [Figure 13-1]). Results obtained with hand-held echography equipment may be included.

The physical examination should be performed by a physician or another study team member with appropriate training to perform the examination.

6.3.4 Vital Signs

Vital signs, including temperature, heart rate, oxygen saturation, respiratory rate and blood pressure, will be measured at baseline, and at all subsequent visits from Day 1 to Day 28 (see Section 13.1 [Table 13-1] and Section 13.2 [Figure 13-1]). Vital signs in ambulant patients will be measured 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 of care, readings from invasive blood pressure monitoring are to be recorded.

On visit days when study drug is administered (Days 1, 2 and 3), vital signs (excluding temperature) will be measured and recorded as follows:

- Immediately before the administration of the study drug
- Within 5 minutes of the start of the study drug infusion
- 30 minutes after the start of the study drug infusion
- Immediately after the completion of the administration of the study drug, which includes post-dose saline flushing
- 30 and 60 minutes after completion of study drug administration

Additionally, on Day 1 only, vital signs will be measured and recorded as follows:

- 2 hours, 3 hours, 4 hours, and 5 hours after completion of study drug administration

At all other visits, the vital signs can be assessed at any moment during the day. The time at which vital signs have been measured will be recorded in the eCRF.

6.3.5 Weight

For determination of study drug dosage, body weight (in kg or lb) will be estimated or measured. The hospital admission weight (in kg or lb) will be used for study drug preparation and for calculation of fluid-corrected serum creatinine.

6.3.6 12-Lead Electrocardiogram

A 12-lead ECG will be performed at baseline, Day 3, and Day 14 (see Section 13.1 [Table 13-1] and Section 13.2 [Figure 13-1]).

A 12-lead ECG with at least a 30-second rhythm strip will be recorded after the patient has rested supine or semi-recumbent for at least 5 minutes. The recording should be printed, signed, and dated by the investigator (or designee) and retained as source documentation. The ECG print should include the date and time of the assessment and the patient's study number. The interpretation of the investigator or designee will be recorded in the source documents.

6.3.7 Laboratory Tests Performed by the Local Laboratory

Blood samples will be collected for local laboratory measurements at baseline, and at visits on Days 1, 3, 5, 7, 14, 21, and 28 (see Section 13.1 [Table 13-1] and Section 13.2 [Figure 13-1]). Approximately 178 to 208 mL (35 to 42 teaspoons) of blood will be collected per patient in total during the study, including tests performed in the local and in the central laboratories.

To decide when to stop RRT (see Section 6.3.9), local urine creatinine, urine volume, and serum creatinine samples will be taken and the creatinine clearance calculated. This will be based on local laboratory results.

The following laboratory tests should be performed at the visits indicated above, unless otherwise indicated:

Clinical chemistry: CRP, ALT, AST, GGT, urea, LDH, creatinine, bilirubin, creatine phosphokinase, total protein, albumin, glucose, sodium, potassium, chloride, bicarbonate, and lactate.

Note: AP is not allowed to be measured locally until Day 14 (see Section 6.3.8.2).

Hematology: hemoglobin, hematocrit, leukocytes, differential leukocytes, erythrocytes, thrombocytes, activated partial thromboplastin time.

Pregnancy test: only at baseline. Blood human chorionic gonadotropin (hCG) or urine hCG (dipstick). See Section 6.4 for additional details.

When the diagnosis of AKI was made according to one of the AKIN serum creatinine criteria (absolute or relative increase, see inclusion criteria 5a and 5b), the confirmative fluid-corrected serum creatinine sample with results available prior to randomization and prior to study drug administration is to confirm continuation of AKI.

Note that platelets, bilirubin, creatinine, and urine output are required to obtain a SOFA score.

6.3.8 Laboratory Tests Performed by Central Laboratory

Blood and urine samples for laboratory tests at baseline and at visits from Day 1 to Day 90 as shown in Section 13.1 [Table 13-1] and Section 13.2 [Figure 13-1] will be collected and forwarded to the central laboratory for analysis. Samples will be processed, stored (if necessary), and shipped according to the instructions provided in the central laboratory manual.

Serum and urine samples for creatinine, urea, proteinuria, AP activity (baseline), sodium, tubular injury biomarkers, purines, systemic markers for inflammation, PK, serology, and anti-drug antibodies will be collected and shipped to the central laboratory.

For selected sites only, detailed instructions for iohexol and related measurements are provided in Section 13.5 (Appendix 5).

6.3.8.1 Endogenous Creatinine and Blood Urea Nitrogen Clearance

The efficacy of the treatment will be investigated by calculating the time-corrected endogenous creatinine clearance. Blood urea nitrogen clearance will also be measured. To calculate the time-corrected endogenous creatinine clearance, timed urine (including measurement of volume) and blood samples need to be collected.

Urine and serum creatinine samples will be collected at all visits from baseline to Day 7 and shipped to the central laboratory. Two serum creatinine samples, one right before the start and one after the stop of the 6 ± 1 hour urine collections have to be collected. Urine volume, urinary creatinine, and the average of two serum creatinine measurements will be used for the calculation of time corrected endogenous creatinine clearance at all visits from baseline to Day 7, and will be calculated by the central laboratory. The measurements from Day 1 to Day 7, inclusive, will be used to calculate endogenous creatinine clearance, the primary endpoint. Only if reliable urine collection is possible, urine will be collected on Days 14, 21, and 28 for time-corrected endogenous creatinine clearance (i.e., patient is in the ICU or intermediate care unit or hospital). If reliable urine collection is not possible, one serum creatinine sample still needs to be taken for calculating eGFR (see Section 13.1 [Table 13-1] and Section 13.2 [Figure 13-1]).

Urine must be collected daily for a period of 6 ± 1 hour at baseline and from Day 1 to Day 7.The collection period will take a minimum of 5 hours, and a maximum of 7 hours. The exact duration of urine collection period and of volume collected will be recorded in the eCRF. The volume should be corrected to account for the volume of samples previously taken from the total urine initially collected.

Endogenous creatinine and BUN clearance will be calculated as follows:

$$\text{clearance} = (\text{urine volume} \times \text{concentration in urine}) / \text{concentration in serum}$$

Note: when patients during the study leave the ICU or intermediate care unit, but are still in the hospital, attempts should be made to continue with the 6-hour urine collection. 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, a blood sample will be drawn at this time too. The urine volume produced over the last approximately 6 hours will be entered in the eCRF. When patients are discharged from the hospital, 6-hour urine collection at home is not considered feasible or reliable and, thus, will not be performed.

6.3.8.2 Alkaline Phosphatase Activity

Alkaline phosphatase activity will only be measured at baseline (pre-dose). Alkaline phosphatase activity results from blood samples taken during the first 14 days of the study are not to be reported to the study team members or to any other study staff member involved in the conduct of the study as it could lead to unblinding and to erroneous interpretation of liver function, as the recAP administered will increase the AP activity (see Section 5.6).

6.3.8.3 Pharmacokinetic Assessments

Pharmacokinetic assessments will be performed during Part 1 only.

The following PK samples will be taken from Day 1 to Day 7 (inclusive) in the first 120 patients from Part 1 only and exact sampling clock times will be recorded:

- Day 1: One pre-dose sample and one post-dose sample near peak (approximately 2.5 ± 1.0 hour after dosing).
- Day 2: One sample near trough (1.5 ± 1 hour before the second dose) and one sample near peak (approximately 2.5 ± 1.0 hour after Day 2 dosing).*
- Day 3: One sample near trough (1.5 ± 1 hour before the third dose) and one sample near peak (approximately 2.5 ± 1.0 hour after Day 3 dosing).*
- Day 4: One PK sample, random time.
- Day 5: One PK sample, random time.
- Day 6: One PK sample, random time.
- Day 7: One PK sample, random time.

6.3.8.4 Immunogenicity Assessments

The following immunogenicity assessments will be performed:

- IgG, IgE, and total immunoglobulin
- Anti-drug antibodies

Blood samples for IgG, IgE, and total immunoglobulin assessment will be taken at baseline and on Days 14 and 28 (see Section 13.1 [Table 13-1] and Section 13.2 [Figure 13-1]).

Blood samples for anti-drug antibodies assessment will be taken at baseline and on Days 14, 28, 60, and 90 (see Section 13.1 [Table 13-1] and Section 13.2 [Figure 13-1]). Samples taken on Days 60 and 90 will only be analyzed in case of a positive result on Day 28; however, as the results for Day 28 will not be available before Day 60, blood samples for anti-drug antibodies will be taken from all patients on Days 60 and 90. In case the results are negative on Day 14 and Day 28, samples taken on Days 60 and 90 will not be analyzed.

Assays for the determination of levels of circulating recAP (Section 6.3.8.3) and anti-drug antibodies were previously qualified in normal human serum samples according to validation procedures and acceptance of the Food and Drug Administration (FDA) Guidance for Industry, EMA guidelines and ICH guidelines for use in Phase 1 clinical trial (DHHS 2001, DeSilva et al 2003; EMA 2006; Neyer et al 2006; Shankar et al 2006; Viswanathan et al 2007; EMA 2007; EMA 2009; EMA 2011; ICH 2011). Additional qualification studies for both assays have been successfully completed in serum from sepsis patients under study plan AMF2013EL-14213-A for the determination of levels of circulating recAP and study plan AMF2013EL-14213-C for the determination of anti-drug antibodies, to support the use of the assays in the Phase 2 clinical study (AP-recAP-AKI-02-01).

6.3.8.5 Biomarker Assessments

Kidney function markers (e.g., urine and serum creatinine, BUN/urea clearance, sodium, proteinuria, fractional excretion of sodium and urea, and urine output), tubular injury biomarkers (e.g., KIM-1, IL-18, GST-alpha), and biomarkers for systemic inflammation (e.g., IL-6, CRP, LBP) will be measured during the duration of the study. Blood and urine samples will be collected at baseline, and at all visits from Day 1 to Day 28 (see Section 13.1 [Table 13-1] and Section 13.2 [Figure 13-1]).

Blood samples will be collected for the systemic inflammatory biomarkers: e.g., Il-6, CRP, and LBP.

Urine samples will be collected for the renal damage biomarkers: KIM-1, IL-18 and GST-alpha.

Any blood and urine samples not used for the biomarker assessments described will be stored by the central laboratory for a maximum of 1 year after the finalization of the study report to be possibly analyzed in an exploratory manner, in case new biomarkers are developed during the conduct of the study.

6.3.8.6 Pharmacodynamic Assessments

For a limited number of sites only, a urine sample for PD assessment will be taken from the 6 ± 1 hour urine collection see Section 13.5 (Appendix 5).

6.3.8.7 Glomerular Filtration Rate

A limited number of sites only will simultaneously determine GFR with iohexol administration and blood sampling (considered the golden standard) to globally compare and assess correlation of GFR and calculated endogenous creatinine clearance. In addition, at these sites samples from the effluent from patients on RRT will be taken to assess exogenous (machine) creatinine clearance. Detailed instructions to these sites for iohexol and exogenous creatinine clearance are provided in Section 13.5.

6.3.9 Renal Replacement Therapy

From Day 1 to Day 7 only continuous modalities of RRT are allowed. From Day 8 onward, intermittent/non-continuous modalities such as intermittent hemodialysis are allowed.

Initiation of RRT: Initiation of RRT should be based on the criteria described by Bellomo et al (2012).

Meeting at least one criterion of the below makes the patient eligible for initiation of RRT:

1. Anuria (negligible urine output for 6 hours)
2. Severe oliguria (urine output < 200 mL over 12 hours)
3. Hyperkalemia (potassium concentrations > 6.5 mmol/L)
4. Severe metabolic acidosis (pH < 7.2 despite normal or low partial pressure of carbon dioxide in arterial blood)

5. Volume overload (especially pulmonary edema unresponsive to diuretics)
6. Pronounced azotemia (urea concentrations > 30 mmol/L or creatinine concentrations > 300 µmol/L)
7. Clinical complications of uremia (e.g., encephalopathy, pericarditis, neuropathy)

Timing of Stopping RRT: Termination of dialysis is based on criteria as used in the VA/NIH Acute Renal Failure Trial Network Study (VA/NIH Acute Renal Failure Trial Network, 2008). If (on continuous renal replacement therapy or between intermittent hemodialysis sessions) diuresis > 30 mL/hour and there are no other indications for RRT, then endogenous creatinine clearance should be calculated using a 6-hour urine collection period. If endogenous creatinine clearance ≥ 20 mL/min, RRT should be discontinued. If endogenous creatinine clearance ≤ 12 mL/min, RRT should be continued. If endogenous creatinine clearance > 12 mL/min and < 20 mL/min, continuation or termination will be the decision of the treating physician. Exact criterion or criteria, corresponding values (e.g., sodium, potassium, or pH) and deviations (e.g., RRT not started when indicated or RRT started when not indicated) need to be meticulously recorded in the eCRF.

However, although the criteria for starting and stopping mentioned above are strongly preferred within the protocol setting, based on clinical judgment investigators may deviate from these criteria. For example, several individual criteria might just not meet the required threshold, but together could provide a valid clinical reason to start RRT. It is also possible that an investigator decides not to initiate RRT based on clinical judgment, although starting criteria might be met. When RRT is initiated despite not meeting criteria, or when RRT is not initiated despite meeting criteria, this should be recorded and a reason for the clinical decision should be provided in the eCRF. Similarly, deviations from the protocol-defined stopping criteria, should be recorded in the CRF, together with the reason for the deviation. A worksheet will be provided as source to record decisions on initiation and termination RRT and deviations.

The following data will be collected in the eCRF:

- Requirement for RRT (over study period): RRT initiated (yes or no)
- Duration of RRT: Exact initiation and termination dates, times of dialysis, and reasons to initiate and terminate RRT. Temporary stopping of RRT need not be recorded (e.g., for filter failures or procedures requiring transportation such as computed tomography scan or others)
- Incidence of dialysis dependency at Day 60 and Day 90.

6.3.10 Lung Function Assessments

Method of ventilation, e.g., non-rebreathing mask, non-invasive mechanical ventilation, invasive mechanical ventilation, will be recorded in the eCRF.

Details on mechanical ventilation will be measured to assess the patient's dependency on mechanical ventilation.

If the patient requires mechanical ventilation (invasive or non-invasive), the start and stop day and time will be recorded in the eCRF in order to calculate the number of days in which mechanical ventilation is needed. If modes of mechanical ventilation are changed (e.g. invasive mechanical ventilation is replaced with non-invasive continuous positive airway pressure (CPAP), a new eCRF page needs to be completed for new mode of mechanical ventilation.

During mechanical ventilation, arterial PaO_2, FiO_2, and PEEP, and during invasive mechanical ventilation only tidal volume will be captured as estimated daily averages at baseline and at all visits from Day 1 to Day 28 (see Section 13.1 [Table 13-1] and Section 13.2 [Figure 13-1]).

The fraction PaO_2 /FiO_2 will be measured to assess the degree of severity of lung function damage. Fraction PaO_2/FiO_2 will be calculated based on values which must be captured at the same time point. Only one simultaneous measurement will be used (i.e., do not use daily average). In case of multiple measurements of this fraction the worst value must be used.

6.3.11 Scoring Systems

6.3.11.1 Acute Physiology and Chronic Health Evaluation Score

The APACHE II score is a severity-of-disease classification system, used in ICU or intermediate care unit settings. The score from 0 to 71 is computed on 12 physiological measurements. The higher the score the more severe the disease and higher the risk of death.

The APACHE II score will be assessed at baseline (i.e., not necessarily at ICU or intermediate care unit admission) and recorded in the eCRF.

6.3.11.2 Simplified Acute Physiology Score 2

The SAPS-2 score is another severity-to-disease classification system, based upon 17 variables, including 12 physiological parameters.

The SAPS-2 score will be assessed at baseline and recorded in the eCRF.

6.3.11.3 Sequential Organ Failure Assessment Score

The SOFA score provides a tool to quantify the severity of the patient's illness, based on the degree of organ dysfunction. The SOFA score is used to follow the condition of the patients in terms of organ failure over time.

The SOFA score will be assessed at baseline, and at all planned visits from Day 1 to Day 28 (as long as the patient is in the ICU or intermediate care unit) and at discharge from ICU or intermediate care unit (see Section 13.1 [Table 13-1] and Section 13.2 [Figure 13-1]). Results will be recorded in the eCRF. Note that platelets, bilirubin, creatinine, and urine output are required to obtain a SOFA score.

6.3.11.4 Quality of Life

The EQ-5D questionnaires will be performed at baseline, at discharge from ICU or intermediate care unit, and at Day 90 (see Section 13.1 [Table 13-1] and Section 13.2 [Figure 13-1]).

The EQ-5D covers 5 dimensions of general well-being. The questionnaire consists of 1 page with 5 questions and 1 page with an analog scale. The questionnaire should be completed by the patient. If the patient is not able to complete the questionnaire himself or herself (e.g., at baseline), it can be completed by a relative.

6.3.12 Concomitant Medication

Concomitant medication will be checked at each visit and recorded in the eCRF. Concomitant medication will be recorded at screening (verification that no concomitant medications that should be avoided are taken), and from baseline up to Day 28, inclusive (see Section 13.1 [Table 13-1] and Section 13.2 [Figure 13-1]).

For continuously intravenously administered medication (e.g., insulin, vasopressors, inotropics, diuretics, sedatives, metoprolol, and potassium), only the name, estimated mean daily dose or mean daily dose as calculated by the system will be recorded, and stop and start dates should be recorded in the eCRF for those patients who are in the ICU or intermediate care unit (see Section 5.7).

6.3.13 Other Collected Data

The following data will also be collected during the study:

Number of shock-free days: measured with dates and times of treatment with inotropic agents or vasopressors – day and hour

Length of ICU or intermediate care unit stay: the admission date and planned and actual discharge date of the patient will be recorded.

Length of hospital stay: the admission date and planned and actual discharge date of the patient will be recorded. Reason for hospitalization will also be recorded.

6.3.14 Adverse Events

The investigator is responsible for reporting all treatment-emergent AEs (TEAEs) that are observed or reported during the study, regardless of their relationship to study drug or their clinical significance.

6.3.14.1 Definitions of Adverse Events

An AE is defined as any untoward medical occurrence (e.g., symptom, sign, diagnosis or diagnostic test finding) in a patient enrolled into a clinical study regardless of its causal relationship to study drug.

A 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.

An SAE is defined as any event that results in death, is immediately life threatening, requires inpatient hospitalization or prolongation of existing hospitalization, results in persistent or significant disability/incapacity, or is a congenital anomaly/birth defect. Important medical events that may not result in death, be life threatening, or require hospitalization may be

considered SAEs when, based upon appropriate medical judgment, they may jeopardize the patient or may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in other situations, such as important medical events that may not be immediately life threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the other outcomes listed in the aforementioned definition. These should also be considered serious. A death occurring during the study or which comes to the attention of the investigator until Day 28 visit, whether considered treatment related or not, must be reported as an SAE. In the event of an SAE the investigator may immediately stop treatment (during the treatment phase, i.e., the first 3 days of the study) if it is considered in the best interest of the patient. In case study drug is discontinued early, the patient will continue follow-up in the study as per protocol. If interrupted for any reason, re-starting of study drug should be discussed with the Medical Monitor (see Section 4.2.1).

Any medical condition that is present at the time that the patient is screened but does not deteriorate should not be reported as an AE. However, if the medical condition deteriorates at any time during the study, it should be recorded as an AE.

6.3.14.2 Eliciting and Documenting Adverse Events

Adverse events will be collected and assessed from the time the patient signs the ICF until Day 28 visit for all AEs (including SAEs) regardless of its relationship to study drug. Any AE that occurs during or after the first dose of study drug is considered treatment emergent.

At every study visit, patients will be asked a standard non-leading question to elicit any medically related changes in their well-being. At follow-up visits, they will also be asked if they have been admitted to the hospital, had any accidents, used any new medications, or changed concomitant medication regimens.

When patients have left the hospital they will be instructed to contact the investigator at any time if any symptoms develop.

Adverse events and SAEs that occur after Day 28 visit, will be reported to PPD or sponsor, only if the investigator considers them possibly, probably, or definitely related to study drug.

In addition to patient accounts, AEs will be identified through physical examination, review of laboratory values, diagnostic test findings (Section 6.5) and monitoring in the ICU/ healthcare facility and (if relevant) report from carers..

6.3.14.3 Reporting Adverse Events

All AEs reported or observed after the patient signs the ICF until Day 28 visit will be recorded on the AE page of the eCRF. Information to be collected includes event term, time of onset, investigator-specified assessment of severity and relationship to study drug, date of resolution of the event, seriousness, any required treatment or evaluations, and outcome. Adverse events resulting from concurrent illnesses, reactions to concurrent illnesses, reactions to concurrent medications, or progression of disease states must also be reported. All AEs will be followed to adequate resolution. The Medical Dictionary for Regulatory Activities (MedDRA) will be used to code all AEs.

Any AE that meets SAE criteria (see Section 6.3.14.1) must be reported to the PPD Pharmacovigilance Department immediately (i.e., within 24 hours) after the time site staff first learn about the event. The following contact information is to be used for SAE reporting:

PPD Pharmacovigilance Department

SAE Hotline United States:	**1-888-483-7729**
SAE Fax line United States:	**1-888-529-3580**
SAE Hotline European Union:	**+44 (0) 1223374240**
SAE Fax line European Union:	**+44 (0) 1223374102**

6.3.14.4 Assessment of Severity

The severity of an AE refers to the extent to which an AE affects the patient's daily activities. The severity of the AE 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.

When changes in the severity of an AE occur more frequently than once a day, the maximum severity for the event should be noted for that day. Any change in severity of signs and symptoms over a number of days will be captured by recording a new AE, with the amended severity grade and the date (and time, if known) of the change. Changes in the severity of an AE should be documented to allow an assessment of the duration of the event at each level of intensity to be performed.

6.3.14.5 Assessment of Causality

The assessment of the relationship of an AE to the administration of study drug (unrelated, possible, probable, definite) is a clinical decision based on all available information at the time of completion of the eCRF. As per the International Council for Harmonisation harmonised tripartite guideline E6(R1): Good Clinical Practice (ICH E6[R1]) guidelines, factors to be considered include the following:

- Temporal sequence from drug administration: the event must occur after the study drug is given. The length of time from exposure to medication to event should be evaluated in the clinical context of the event.
- Recovery on discontinuation (dechallenge) and recurrence on reintroduction (rechallenge).
- Underlying, concomitant, intercurrent diseases: each report should be evaluated in the context of the natural history and course of the disease being treated and any other disease the patient may have had prior to, or developed during the course of the study.
- Concomitant medication or treatment: the other drugs the patient is taking or the treatment the patient is receiving at the time of the event should be examined to determine whether any of them may be recognized to cause the event in question.
- The pharmacology and PK of the study drug: absorption, distribution, metabolism, and excretion of the study drug or other medications the patient is receiving coupled with the PD responses should be considered when evaluating an event.

The investigator’s assessment of an AE’s relationship to study drug is part of the documentation process. If there is any doubt as to whether a clinical observation is an AE or whether it is related to study drug, the event should be reported.

The relationship or association of the study drug in causing or contributing to the AE 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.

6.3.14.6 Follow-up of Patients Reporting Adverse Events

All AEs must be reported in detail on the appropriate page of the eCRF and followed to satisfactory resolution, until the investigator deems the event to be chronic or not clinically significant, until the patient is considered to be stable or subject is lost to follow-up.

6.4 Pregnancy

The effect of recAP on pregnancies or infants is not known, as the safety of this drug during pregnancy has not been tested previously in either human or in animal studies. Because AP is present in the human placenta it is theoretically possible that anti-placental antibodies are developed after receiving study drug. Such antibodies could interfere with the ability to have a successful pregnancy. While development of anti-drug antibodies has not been seen to date in human studies, the safety experience with this drug is limited to only 37 subjects who received study drug and no data are available regarding effect of the drug on human reproduction.

Therefore, female patients who are pregnant or lactating/breastfeeding at time of screening or who intend to become pregnant within 28 days of enrolling into the study will be excluded from the study (see Section 4.1.2). If a pregnancy occurs while a patient is participating in the study, it is not regarded as an AE unless there is a suspicion that an investigational product may have interfered with the effectiveness of a contraceptive medication. Any pregnancy that occurs during study participation must be reported using a clinical study pregnancy form. To ensure patient safety, each pregnancy must be reported to AM-Pharma B.V. within 2 weeks of learning of its occurrence. The pregnancy must be followed-up to determine outcome (including spontaneous miscarriage, elective termination, normal birth, or congenital abnormality) and status of mother and child, even if the patient was discontinued from the study. Pregnancy complications and elective terminations for medical reasons should not be reported as an AE or SAE. Spontaneous miscarriages must be reported as SAEs.

Any SAE occurring in association with a pregnancy, brought to the investigator's attention after the patient has completed the study, and considered by the investigator as possibly related to the study drug, must be promptly reported to AM-Pharma B.V.

6.5 Laboratory Analyses

Any abnormal laboratory test results (hematology, clinical chemistry, or urinalysis) or other safety assessments (e.g., ECGs, vital sign measurements), including those that worsen from baseline, felt to be clinically significant in the medical and scientific judgment of the investigator, are to be recorded as (S)AEs.

7 Statistical and Analytical Plan

7.1 Primary Endpoint

The primary endpoint will be calculated from time-corrected endogenous creatinine clearance measurements on Day 1 (first measurement after treatment) to Day 7, inclusive. Time-corrected 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. 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 time-corrected endogenous creatinine clearance

values over the 7 days. Specifically, denoting C_i as the mean time-corrected endogenous creatinine clearance on Day i, AUC_{1-7} is defined as:

$$AUC_{1-7} = \frac{1}{7}\sum_{i=1}^{7} C_i$$

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 (i.e., prior to treatment) will be carried forward.

7.2 Key Secondary 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. The analysis of this endpoint will be repeated excluding patients who died or withdrew from the study prior to completion of this period.

7.3 Other Secondary Endpoints

7.3.1 Renal

- Renal endpoints include the following:
- Volume of urine (daily from Day 1 to Day 7, inclusive, and on subsequent visit days if reliable urine collection is possible)
- Serum creatinine and BUN (daily from Day 1 to Day 7, inclusive, and on subsequent visit days if reliable urine collection is possible)
- BUN 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 BUN (during the period Day 1 to Day 7, inclusive)
- RRT-free days (during the period Day 1 to Day 28 excluding patients who died or withdrew from the study prior to the end of this period), i.e., 28 days minus the number of days a patient is on RRT during this period. An RRT-free day is defined as 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:

a. 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

b. 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 on 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 did receive any form of RRT 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 28 as assessed by measured creatinine clearance if available, otherwise as assessed by eGFR (estimated by the CKD-EPI formula based on serum creatinine). Patients who die prior to Day 28 will be excluded from this endpoint, with any other missing assessments (including early withdrawals) imputed using LOCF.
- Kidney function at Day 60 and Day 90 as assessed by eGFR (estimated by the CKD-EPI formula based on serum creatinine). Patients who die prior to Day 60 or Day 90, respectively, will be excluded from this endpoint, with any other missing assessments (including early withdrawals) imputed using LOCF.
- 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). Patients who die prior to Day 60 or Day 90, respectively, will be excluded from this endpoint, with any other missing assessments (including early withdrawals) at Day 60 or Day 90 imputed using LOCF.
- Incidence of dialysis dependency 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 dialysis dependency will be counted as not having required dialysis dependency.

7.3.2 Other Organs

Endpoints for organs other than renal function include the following:

- Liver enzymes (AST, ALT, GGT, LDH, bilirubin but excluding AP).
- Lung function as assessed by arterial PaO_2, FiO_2, P/F ratio, PEEP, and tidal volume in mechanically ventilated patients.
- Ventilator-free days (from Day 1 to Day 28, inclusive), i.e., 28 days minus the number of days a patient is on ventilator during this period. 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.
- Time from start of first administration of study drug to being off-ventilator (from Day 1 to Day 28, inclusive) for those patients who are on ventilator at the start of this period. Patients who remain on ventilator will be censored at the earliest of Day 28 or withdrawal from the study for any reason.
- Shock-free days (during the period Day 1 to Day 28, inclusive), i.e., 28 days minus the number of days a patient is on inotropic or vasopressor therapy during this period. 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. 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, and 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. Patients who do not become shock free will be censored at the earliest of Day 28 or withdrawal from the study for any reason.
- SOFA scores during ICU or intermediate care unit stay.
- Number of dysfunctional organs as assessed by SOFA scores.
- Deaths during the 90-day study period (by recording date).

7.3.3 Biomarkers

Biomarker endpoints include the following:

- Kidney function markers (e.g., urine and serum creatinine, BUN/urea clearance, sodium, proteinuria, fractional excretion of sodium, urea, and urine output).
- Tubular injury biomarkers (e.g., KIM-1, IL 18, GST-alpha).
- Biomarkers for systemic inflammation (e.g., IL-6, CRP, LBP).
- Pharmacokinetics of recAP in all 3 active treatment groups in the first 120 patients from Part 1 only
- In addition to recAP PK concentration measurements, baseline (pre-dose) AP will be measured by activity (central laboratory)

7.3.4 Other Endpoints

Additional endpoints include the following:

- Composite endpoints – patients that meet, or do not meet, at least one of the following criteria:
  a. Received RRT or died (prior to Day 28 [inclusive])
  b. eGFR < 60 mL/min (at Day 60, estimated by a commonly used formula such as MDRD or CKD-EPI), or requiring chronic RRT, or died (prior to Day 60)
  c. eGFR < 60 mL/min (at Day 90, estimated by the CKD-EPI formula based on serum creatinine), or requiring chronic RRT, hospitalized for a new episode of AKI (at Day 90), or died (prior to Day 90)
- Serology as assessed by IgG, IgE, and total immunoglobulin
- Safety parameters including (S)AEs, laboratory assessments (clinical chemistry, hematology, and urinalysis parameters not considered in the efficacy analysis), vital signs, and ECG data
- Quality of life, assessed by the EQ-5D questionnaire at baseline, ICU or intermediate care unit discharge, and Day 90
- Time from start of first administration of treatment to discharge from ICU or intermediate care unit (during the period Day 1 to Day 28, inclusive, for patients in ICU or

intermediate care unit at the start of this period) 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 or intermediate care unit from the start of first administration of study drug (during the period Day 1 to Day 90, inclusive) using the times when the decision is made to admit or discharge the patient as appropriate
- Time from start of first administration of treatment to discharge from hospital (during the period Day 1 to Day 90, inclusive) 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 90, inclusive) using the times when the decision is made to admit or discharge the patient as appropriate

7.4 Sample Size Calculations

A sample size of $n_1 = 30$ patients per treatment group in Part 1 with an additional $n_2 = 85$ patients recruited to 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 may exceed 290 patients. Patients recruited during this period to the dose selected in Part 2 will form part of the Part 2 populations, but those recruited to the doses that are not selected will be included in the Part 1 population. Custom programmed simulations were performed using SAS software Version 9.2 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 ($AUC_{1\text{-}7}$) 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.

7.5 Analysis Sets

The following analysis sets will be used in the statistical analyses:

- **All enrolled set**: all patients enrolled in the study. Patients will be analyzed according to the treatment they were randomly assigned to, with a "Not randomly assigned" treatment group included if required.

- **ITT combined set**: all patients who were randomly assigned to a study drug. 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.

- **ITT Part 1 set**: all patients who were randomly assigned to a study drug prior to the conclusion of Part 1 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.

- **ITT Part 1 interim set**: 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.

- **ITT Part 2 set**: all patients who were randomly assigned to a study drug after the conclusion of Part 1 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.

- **Per-protocol (PP) Day 1-7 combined set**: all patients who were randomly assigned to a study drug, had no significant protocol deviations (e.g., wrong drug volume received, administration of prohibited concomitant medication, patient did not meet all inclusion criteria or met 1 or more exclusion criteria) and had complete data 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 and the Significant Protocol Deviation Rules document for further information on the handling of protocol deviations for this study.

- **Per-protocol (PP) Day 1-7 Part 2 set**: all patients who were randomly assigned to a study drug after the conclusion of Part 1 of the study, had no significant protocol deviations (e.g., wrong drug volume received, administration of prohibited concomitant medication, patient did not meet all inclusion criteria or met 1 or more exclusion criteria) and had complete data 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 and the Significant Protocol Deviation Rules document for further information on the handling of protocol deviations for this study.

- **PP Day 1-28 set**: all patients who were randomly assigned to a study drug, had no significant protocol deviations (e.g., wrong drug volume received, administration of prohibited concomitant medication, patient did not meet all inclusion criteria or met 1 or more exclusion criteria) and had complete data for the key secondary endpoint. The key secondary endpoint will be analyzed for the PP Day 1-28 set, with patients analyzed according to the treatment they received. See Section 10.2.2 and the Significant Protocol Deviation Rules document for further information on the handling of protocol deviations for this study.

- **Safety set**: 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.

- **PK set**: 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.

7.6 Description of Subgroups to Be Analyzed

To determine whether patient groups that benefit most from recAP treatment and whether patient groups that are non-responders can be identified, additional analyses will be performed on a selection of endpoints (AUC_{1-7}; RRT incidence from Day 1 to Day 28,

inclusive; peak value of serum creatinine from Day 1 to Day 7, inclusive; RRT-free days from Day 1 to Day 28, inclusive; time to discharge from ICU or intermediate care unit from Day 1 to Day 28, inclusive; deaths during the 90-day study period) for subgroups based on the following parameters:

- Baseline characteristics, including:
    - Kidney function markers (e.g., urine and serum creatinine, BUN/urea clearance, sodium, proteinuria, fractional excretion of sodium and urea, and urine output)
    - Tubular injury biomarkers (e.g., KIM-1, IL-18, GST-alpha)
    - Biomarkers for systemic inflammation (e.g., IL-6, CRP, LBP)
    - Glomerular filtration rate (eGFR by chronic kidney disease epidemiology collaboration [CKD-EPI])
    - APACHE II score (<25, ≥25)
- Timing from first diagnosis of SA-AKI to start of recAP treatment, e.g., in time intervals (0 to < 6 hours, 6 to < 12 hours, 12 to < 18 hours, 18 to < 24 hours).
- Baseline AKIN stage (stage 1, stage 2, stage 3)

Additional parameters may also be considered (e.g., urine output [over or under 200 mL/day], diabetes mellitus [yes or no], hypertension [yes or no], age [< 55 years old, ≥ 55 years old and < 70 years old, or ≥ 70 years old], sex (male or female), risk scores [high or low]).

Further details of these subgroups, including cut-off points and relevant outputs to be produced will be provided in the SAP.

7.7 Statistical Analyses Methodology

Statistical analyses will be performed using SAS software Version 9.2 or later. Continuous variables will be summarized using descriptive statistics, including but not limited to the mean, the standard deviation, median, minimum value, and maximum value. Categorical variables will be summarized using frequency counts and percentages. All data collected across the 4 treatment groups throughout the study will be listed in data listings.

All statistical tests will be 2-sided and performed using a 5% significance level, leading to 95% (2-sided) CIs. 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 7.7.1). 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.

The 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 (for all 4 treatment groups) will be repeated for Part 1 and Part 2. If any notable differences are observed, then the combined analysis of Parts 1 and 2 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 combined analysis of Parts 1 and 2 being interpreted with caution and further exploratory analyses conducted to investigate the possible causes.

Further details of the statistical analyses, methods, and data conventions will be described in the SAP.

7.7.1 Analysis of Primary Efficacy Endpoint

The primary efficacy endpoint is the area under the time-corrected endogenous creatinine clearance curve from Day 1 to Day 7, i.e., $AUC_{1\text{-}7}$.

Area under the time-corrected endogenous creatinine clearance curve from Day 1 to Day 7 will be summarized using descriptive statistics and analyzed using an analysis of variance (ANOVA) with treatment and site as explanatory variables. The analysis will be performed separately for Parts 1 and 2: for Part 1, $AUC_{1\text{-}7}$ will be compared between the 3 recAP doses and placebo. This analysis will be considered in conjunction with the safety data to determine

the optimal recAP dose for use in Part2. For Part 2, the optimal recAP dose will be compared with 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 level α if it and all intersection hypotheses involving it are all rejected at local level α. 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 Parts 1 and 2, respectively, $n = n_1 + n_2$, Φ refers to the standard normal distribution, and p_1 and p_2 are the p-values from Parts 1 and 2, respectively. For the single hypothesis, p_1 is the unadjusted p-value to compare optimal dose with placebo in Part 1. For the intersection hypotheses, p_1 is the Dunnett-adjusted p-value in Part 1, and p_2 is the unadjusted p-value to compare optimal dose with placebo in Part 2 for all hypotheses.

The difference in least squares means between the treatment groups will also be presented along with the associated 95% CI.

The underlying assumptions for ANOVA (normality and homogeneity of variance of the studentized residuals) will be investigated for Part 1 by examining a normal probability plot of the residuals and a plot of the fitted values against the residuals. 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 Parts 1 and 2 (i.e., using ANOVA with the rank-transformed values of $AUC_{1\text{-}7}$ as the outcome variable and treatment and site as explanatory variables).

In addition to the above, the summarization using descriptive statistics will be repeated for all 4 treatment groups for the patients randomly assigned prior to Part 2.

7.7.2 Analyses of Secondary Efficacy Endpoints

Analyses of the secondary efficacy endpoints will be conducted at the conclusion of Part 2.

The key secondary endpoint is the incidence of RRT from Day 1 to Day 28, inclusive with patients who die or withdraw from the study during this period without recording RRT incidence being counted as having not required RRT. This endpoint will be summarized by treatment group using counts and percentages. Should a statistically significant result be obtained from the combination test analysis of the primary endpoint, the optimal recAP dose will be formally compared with placebo using a logistic regression model with treatment group and site as explanatory variables. Otherwise the results will be reported as exploratory analyses only. The odds ratio and associated 95% confidence interval from this comparison will also be presented.

The above summarization and analysis will also be repeated, excluding those patients who die or withdraw from the study from Day 1 to Day 28, inclusive. Should statistically significant results not be obtained from both the primary endpoint and the key secondary endpoint, the results from the logistic regression model for this comparison will be reported as exploratory analyses only.

Descriptive statistics will be used for all other continuous secondary efficacy endpoints, summarizing absolute values and/or change from baseline as required across all four treatment groups in tables and/or figures as appropriate. Categorical secondary efficacy endpoints will be summarized for the treatment groups by counts and percentages, and shift tables as appropriate. Selected endpoints will be analyzed by appropriate methods; these analyses will be exploratory only and defined in more detail in the SAP.

7.7.3 Safety Analyses

The safety summaries will include all 4 treatment groups, comprising all patients in the safety set.

Treatment-emergent adverse events will be tabulated by treatment group. The number and percentage of patients with at least 1 TEAE will be presented along with a breakdown by system organ class and preferred term, with each patient being counted once at each summarization level. This summary will be repeated for treatment-related TEAEs, TEAEs that lead to study drug discontinuation, by relationship to study drug, by intensity, and for treatment-emergent SAEs.

Laboratory test results (for hematology, clinical chemistry, and urinalysis parameters not considered in the efficacy analyses), vital signs data, and ECG data will be presented in tables summarizing absolute values and change from baseline by treatment group, with shift tables included as appropriate. Other safety data will be listed only.

7.7.4 Pharmacokinetic Analyses

A population PK analysis of plasma concentration-time data will be performed using non-linear mixed-effects modeling. Data from this study may be combined with data from an additional study in healthy adult volunteers and included in an integrated PK analysis. The structural model will contain clearance and volume of distribution as fixed-effect parameters. The intersubject variability in the parameter estimates and the random residual error in the data will be estimated with an appropriate error model. Available patient characteristics will be tested as potential covariates affecting PK parameters. Details of the analysis will be given in a population PK analysis plan and the results of the population PK analysis will be presented in a separate report.

7.7.5 Other Analyses

Information from any additional, baseline, or screening assessments (e.g., disposition of patients, demographics, previous medical history, site of infection and pathogen, APACHE II score, SAPS-2, AP levels) will be listed and, where appropriate, presented by means of descriptive statistics for continuous variables, or frequency counts and percentages for categorical variables.

7.7.6 Interim Analyses

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. Further details on the data required will be specified in the DMC charter and the interim analysis plan. The interim analysis will be conducted on all data collected up to the first 7 days of laboratory data of the 120^{th} patient from Part 1.

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. The type 1 error rate will be controlled by combining the results for the primary efficacy endpoint from the interim analysis (Part 1) and the final analysis (Part 2) using the inverse normal method described in Section 7.7.1. 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.

7.8 Data Quality Assurance

The sites will maintain source documentation and enter patient data into the eCRF as accurately as possible and will rapidly respond to any reported discrepancies. Electronic CRFs are accessed through Medidata Rave® (Medidata Solutions Inc, New York, New York). This electronic data capture system is validated and compliant with US Title 21 Code of Federal Regulations (CFR) Part 11. Each person involved with the study will have an individual user name and password that allows for record traceability. Thus, the system, and subsequently any investigative reviews, can identify coordinators, investigators, and individuals who have entered or modified records, as well as the time and date of any modifications. A quality review of the data will be performed by the site with additional reviews by the clinical monitor through source data verification.

Each eCRF is presented as an electronic copy, allowing data entry by study site staff, who can add and edit data, identify, and resolve discrepancies, and view records. This system provides immediate direct data transfer to the database, as well as immediate detection of discrepancies, enabling site coordinators to resolve and manage discrepancies in a timely manner.

Paper copies of the eCRFs and other database reports may be printed by the investigator. This system provides study site staff, monitors, and reviewers with access to hard copy audits, discrepancy reviews, and investigator comment information.

After all data reviews and query resolutions are complete, the statistical analysis plan approved and signed, and any summary/analysis populations approved, the database will be locked.

7.8.1 Data Management

As part of the responsibilities assumed by participating in the study, the investigator agrees to maintain adequate case histories for the patients treated as part of the research under this protocol. The investigator agrees to maintain accurate eCRFs and source documentation as part of the case histories. These source documents may include diary cards, laboratory reports, etc.

All eCRF information is to be completed. If an item is not available or is not applicable, this fact should be indicated. Blank spaces should not be present unless otherwise directed.

Investigative site staff will enter patient data into the electronic data capture system Medidata Rave. The analysis data sets will be a combination of these data and data from other sources (e.g., laboratory data).

Clinical data management will be performed in accordance with applicable PPD standards and data cleaning procedures to ensure the integrity of the data, e.g., removing errors and inconsistencies in the data. Adverse events and concomitant medication terms will be coded using MedDRA, an internal validated medication dictionary, and concomitant medications will be coded using WHO Drug Dictionary.

After database lock, each study site will receive a CD-ROM containing all of their site specific eCRF data as entered into Medidata Rave for the study, including full discrepancy and audit history. Additionally, a CD-ROM copy of all of the study site's data from the study will be created and sent to the sponsor for storage. PPD will maintain a duplicate CD-ROM copy for their records. In all cases, patient initials will not be collected or transmitted to the sponsor.

8 Ethics

8.1 Independent Ethics Committee or Institutional Review Board

Federal regulations and the ICH E6(R1) guidelines require that approval be obtained from an IRB/ IEC before participation of human patients in research studies. Before study onset, the protocol, informed consent, advertisements to be used for the recruitment of study patients, and any other written information regarding this study to be provided to the patient or the patient's legal guardian must be approved by the IRB/IEC. Documentation of all IRB/IEC

approvals and of the IRB/IEC compliance with ICH E6(R1) will be maintained by the site and will be available for review by the sponsor or its designee.

All IRB/IEC approvals should be signed by the IRB/IEC chairman or designee and must identify the IRB/IEC name and address, the clinical protocol by title or protocol number or both, and the date approval or a favorable opinion was granted.

The investigator is responsible for providing written summaries of the progress and status of the study at intervals not exceeding 1 year or otherwise specified by the IRB/IEC. The investigator must promptly supply the sponsor or its designee, the IRB/IEC, and, where applicable, the institution, with written reports on any changes significantly affecting the conduct of the study or increasing the risk to patients.

8.2 Ethical Conduct of the Study

The study will be performed in accordance with the ethical principles that have their origin in the Declaration of Helsinki, ICH E6(R1), CFR, and all other applicable regulations.

8.3 Patient Information and Consent

Fully informed consent will be obtained before any study-specific procedures are performed. Country-specific regulations regarding the collection of informed consent and personal data will be followed at each site. It is anticipated by the very nature of the study that many patients who will be eligible for this protocol will not be able to give fully informed consent themselves due to various reasons including sedation or unconscious state. In a situation where a patient is unable to provide consent, the patient's legally authorized representative may provide written consent, as approved by the institutional-specific guidelines. Informed consent may be obtained from an independent consulting physician or obtained on the basis of emergency study protocol by the investigator in countries where regulation and institution guidelines permit. This form of consent can only be used when the patient and legally authorized representative are unable to provide consent. In cases where the initial informed consent is obtained from a legal representative, an independent consulting physician, or by the investigator, the patient must also give written informed consent as soon as they are able.

The investigator shall retain the signed original ICF(s) and give a copy of the signed original form to the patient or legal guardian.

9 Investigator's Obligations

The following administrative items are meant to guide the investigator in the conduct of the study but may be subject to change based on industry and government standard operating procedures, working practice documents, or guidelines. Changes will be reported to the IRB/IEC but will not result in protocol amendments.

9.1 Confidentiality

All laboratory specimens, evaluation forms, reports, and other records will be identified in a manner designed to maintain patient confidentiality. All records will be kept in a secure storage area with limited access. Clinical information will not be released without the written permission of the patient (or the patient's legal guardian), except as necessary for monitoring and auditing by the sponsor, its designee, the FDA, the European Medicines Agency, other regulatory national authorities, or the IRB/IEC.

The investigator and all employees and coworkers involved with this study may not disclose or use for any purpose other than performance of the study any data, record, or other unpublished, confidential information disclosed to those individuals for the purpose of the study. Prior written agreement from the sponsor or its designee must be obtained for the disclosure of any said confidential information to other parties.

9.2 Financial Disclosure and Obligations

Investigators are required to provide financial disclosure information to allow the sponsor to submit the complete and accurate certification or disclosure statements required under 21 CFR 54. In addition, the investigator must provide to the sponsor a commitment to promptly update this information if any relevant changes occur during the course of the investigation and for 1 year following the completion of the study.

Neither the sponsor nor PPD is financially responsible for further testing or treatment of any medical condition that may be detected during the screening process. In addition, in the absence of specific arrangements, neither the sponsor nor PPD is financially responsible for further treatment of the patient's disease.

9.3 Investigator Documentation

Prior to beginning the study, the investigator will be asked to comply with ICH E6(R1) 8.2 and Title 21 of the CFR by providing the following essential documents, including but not limited to:

IRB/IEC approval

Original investigator-signed investigator agreement page of the protocol

Form FDA 1572, fully executed, and all updates on a new fully executed Form FDA 1572

Curriculum vitae for the investigator and each sub-investigator listed on Form FDA 1572

Financial disclosure information to allow the sponsor to submit complete and accurate certification or disclosure statements required under 21 CFR 54. In addition, the investigators must provide to the sponsor a commitment to promptly update this information if any relevant changes occur during the course of the investigation and for 1 year after the completion of the study.

IRB/IEC-approved informed consent and any other written information regarding this study that is to be provided to the patient or legal guardian

Laboratory certifications and normal ranges for any local laboratories used by the site, in accordance with 42 CFR 493

9.4 Study Conduct

The investigator agrees that the study will be conducted according to the principles of ICH E6(R1). The investigator will conduct all aspects of this study in accordance with all national, state, and local laws or regulations. Study information from this protocol will be posted on publicly available clinical trial registers before enrollment of patients begins.

9.5 Adherence to Protocol

The investigator agrees to conduct the study as outlined in this protocol in accordance with ICH E6(R1) and all applicable guidelines and regulations.

9.6 Adverse Events and Study Report Requirements

By participating in this study the investigator agrees to submit reports of SAEs according to the timeline and method outlined in the protocol. In addition, the investigator agrees to submit annual reports to the study site IRB/IEC as appropriate.

9.7 Investigator's Final Report

Upon completion of the study, the investigator, where applicable, should inform the institution; the investigator/institution should provide the IRB/IEC with a summary of the study's outcome and the sponsor and regulatory authority(ies) with any reports required.

9.8 Records Retention

Essential documents should be retained until at least 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. These documents should be retained for a longer period, however, if required by the applicable regulatory requirements or by an agreement with the sponsor. It is the responsibility of the sponsor to inform the investigator/institution as to when these documents no longer need to be retained.

9.9 Publications

After completion of the study, the data will be reported at scientific meetings and/or submitted and published in a scientific journal, regardless of the outcome. In these cases, a publication committee will be responsible for these activities and will work with the investigators to determine how the manuscript is written and edited, the number and order of authors, the publication to which it will be submitted, and other related issues.

In principle the company supports publication of results, regardless of the outcome. In order to prevent subgroup publications prior to main publication, data are the property of the sponsor and cannot be published without prior authorization from the sponsor, but data and publication thereof will not be unduly withheld.

10 Study Management

Table 10–1 presents the study administrative details.

Table 10–1 Study Administrative Table

Role	Name/Affiliation/Address
Sponsor	AM-Pharma B.V.
Sponsor Signatory and Sponsor Medical Officer	Jacques Arend MD DiMD Vice President Clinical Development and Chief Medical Officer AM-Pharma B.V. Rumpsterweg 6 3981 AK Bunnik The Netherlands Phone: 31 (0) 302598836
Contract Research Organization	PPD Worldwide Headquarters 929 North Front Street Wilmington, NC 28401-3331 Phone: +910-251-0081
Medical Monitor	European Union: Hanna Orr, MD Associate Medical Director, Pharmacovigilance PPD Office Warsaw (Home Based) 49 Domaniewska Street Trinity Park III; 1st floor 02-672 Warsaw, Poland Phone: +48 781811328 United States: William Crafford, MD, FACC Medical Director, Pharmacovigilance PPD 929 North Front Street Wilmington, NC 28401-3331 Phone: 1-800-201-8725
Study Drug Manufacturing Facilities	**Manufacturing, Preparation, and Dispensing:** Nova Laboratories Ltd Martin House, Gloucester Crescent Wigton Leicestershire, UK Phone: +44 (0) 1162230100 Fax: +44 (0) 1162230101 **Labeling and Shipping:** PPD Ireland Depot Building C, Garycastle Business & Technology Park Athlone Co Westmeath, Ireland Phone: +35 3906460300

Role	Name/Affiliation/Address
Central Laboratories	PPD Global Central Laboratory 2 Tesseneer Drive Highland Heights, KY 41076 Phone: 859-781-8877 PPD Global Central Laboratory Cluster Park Kleine Kloosterstraat 19 B-1932 Zaventem, Belgium Phone: +32 2 725 2127
Laboratory for PK Analyses	PRA Bioanalytical Laboratory Netherlands Early Development Services Westerbrink 3, 9405 BJ Assen, The Netherlands Phone: +31 (0) 592303222 Fax: +31 (0) 592303223
Laboratory for Iohexol Analyses	Biomarkers Core Laboratory O'Brien Center for AKI Research UC San Diego Department of Medicine Division of Nephrology-Hypertension 214 Dickinson Street, San Diego CA 92103, USA CTF-C Building # 205 B, 210 & 211, Hillcrest Medical Center Mail Code 8342, Phone: +1 619 471 0407 Fax: +1 619 543 7769
Laboratory for ATP, ADP, AMP, cAMP, and adenosine analyses	Radboud University Medical Center 149 Farmacologie-Toxicologie Geert Grooteplein 21 6525 EZ Nijmegen, the Netherlands

10.1 Monitoring

10.1.1 External Data Monitoring Committee

An independent DMC will review the results of the interim analysis and select the optimum recAP dose for Part 2 of the study (see Section 7.7.5). The DMC will conduct three additional reviews of the safety data by teleconference once the first 7 days of laboratory data are available for the following: 75 patients in Part 1, 60 patients in Part 2, and 125 patients in Part 2. In each case, the milestone will be patients with at least 7 days of laboratory data. Additional electronic reviews will be conducted of unblinded AE listings after 25, 50 and 100 patients in Part 1 and 30 and 90 patients in Part 2. The DMC can also request ad hoc

reviews. Accumulating safety and efficacy data will be reviewed at predefined milestones during Part 1 and Part 2 of the study by the independent DMC as described in the DMC charter as follows:

- Electronic Review 25 subjects
- Electronic Review 50 subjects
- Teleconference 75 subjects
- Electronic Review 100 subjects
- Face to Face Meeting-interim analysis-120 subjects
- Ad hoc Review- (if needed) between 90-120 additional subjects
- Teleconference 60 additional subjects from Part 2 (at least 180 total)
- Electronic Review 90 additional subjects from Part 2 (at least 210 total)
- Teleconference 125 additional subjects from Part 2 (at least 245 total)
- Ad hoc Review- (if needed) between 125-170 subjects from Part 2 (at least 245-290 total).

10.1.2 Monitoring of the Study

The clinical monitor, as a representative of the sponsor, has the obligation to follow the study closely. In doing so, the monitor will visit the investigator and study site at periodic intervals, in addition to maintaining necessary phone and letter contact. The monitor will maintain current personal knowledge of the study through observation, review of study records and source documentation, and discussion of the conduct of the study with the investigator and personnel.

All aspects of the study will be carefully monitored, by the sponsor or its designee, for compliance with applicable government regulation with respect to current ICH E6(R1) and current standard operating procedures.

10.1.3 Inspection of Records

Investigators and institutions involved in the study will permit study-related monitoring, audits, IRB/IEC review, and regulatory inspections by providing direct access to all study records. In the event of an audit, the investigator agrees to allow the sponsor, representatives of the sponsor, or a regulatory agency (e.g., FDA or other regulatory agency) access to all study records.

The investigator should promptly notify the sponsor and PPD of any audits scheduled by any regulatory authorities and promptly forward copies of any audit reports received to the sponsor.

10.2 Management of Protocol Amendments and Deviations

10.2.1 Modification of the Protocol

Any changes in this research activity, except those necessary to remove an apparent, immediate hazard to the patient, must be reviewed and approved by the sponsor or its designee. Any protocol amendments will be prepared by the sponsor/contract research organization. Protocol amendments will be submitted to the IRB/EC and to regulatory authorities in accordance with local regulatory requirements. Approval must be obtained from the IRB/EC and regulatory authorities (as locally required) before implementation of any changes, except for changes necessary to eliminate an immediate hazard to patients or changes that involve logistical or administrative aspects only (e.g., change in Medical Monitor or contact information).

10.2.2 Protocol Deviations

The investigator or designee must document and explain in the patient's source documentation any deviation from the approved protocol. The investigator may implement a deviation from, or a change of, the protocol to eliminate an immediate hazard to study patients without prior IRB/IEC approval. As soon as possible after such an occurrence, the implemented deviation or change, the reasons for it, and any proposed protocol amendments should be submitted to the IRB/IEC for review and approval, to the sponsor for agreement, and to the regulatory authorities, if required.

A deviation from the protocol is an unintended or unanticipated departure from the procedures or processes approved by the sponsor and the IRB/IEC and agreed to by the investigator. A significant deviation (violation) occurs when there is non-adherence to the protocol by the patient or investigator that may result in an additional risk to the patient or which may confound analysis of study results. Significant deviations can include non-adherence to inclusion or exclusion criteria, enrollment of the patient, or non-adherence to FDA regulations or ICH E6(R1) guidelines, and may lead to the patient being withdrawn from the study (see Section 4.2).

Protocol deviations (and violations) will be documented by the clinical monitor throughout the course of monitoring visits. Investigators will be notified in writing by the monitor of deviations. The IRB/IEC should be notified of all protocol deviations in a timely manner.

10.3 Study Termination

Although AM-Pharma B.V. has every intention of completing the study, AM-Pharma B.V. reserves the right to discontinue the study at any time for clinical or administrative reasons.

The end of the study is defined as the date on which the last patient completes the final assessments (includes follow-up assessments).

10.4 Final Report

Whether the study is completed or prematurely terminated, the sponsor will ensure that the clinical study reports are prepared and provided to the regulatory agency(ies) as required by the applicable regulatory requirement(s). The sponsor will also ensure that the clinical study reports in marketing applications meet the standards of the ICH E6(R1): Structure and content of clinical study reports.

Where required by applicable regulatory requirements, an investigator signatory will be identified for the approval of the clinical study report. The investigator will be provided reasonable access to statistical tables, figures, and relevant reports and will have the opportunity to review the complete study results.

Upon completion of the clinical study report, the sponsor will provide the investigator with the full summary of the study results. The investigator is encouraged to share the summary results with the study patients, as appropriate. The study results will be posted on publicly available clinical study registers.

11 Quality Control and Good Clinical Practice Audits

11.1 Quality Control

Quality control for this study will be performed in compliance with PPD’s standard operating procedures.

11.2 Good Clinical Practice Audits

The ICH E6(R1) and CFR audits for this study will be performed in compliance with PPD's standard operating procedures.

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13 Appendices

13.1 Appendix 1: Schedule of Assessments

Table 13-1 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													
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												

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)**
SOFA score[i]		X	X	X	X	X	X	X	X	X	X	X		
EQ-5D[j]		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		
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		

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)**
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		
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											

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)
Discharge from ICU or intermediate care unit / admission or discharge from hospital[x]	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 5.a and 5.b), or according to the AKIN urine output criteria (urinary output < 0.5 mL/kg/h for > 6 hours, see inclusion criterion 5.c), 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 13.2, Figure 13-1) 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 5.a and 5.b), 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 5.c), 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 6.3.8.3 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.

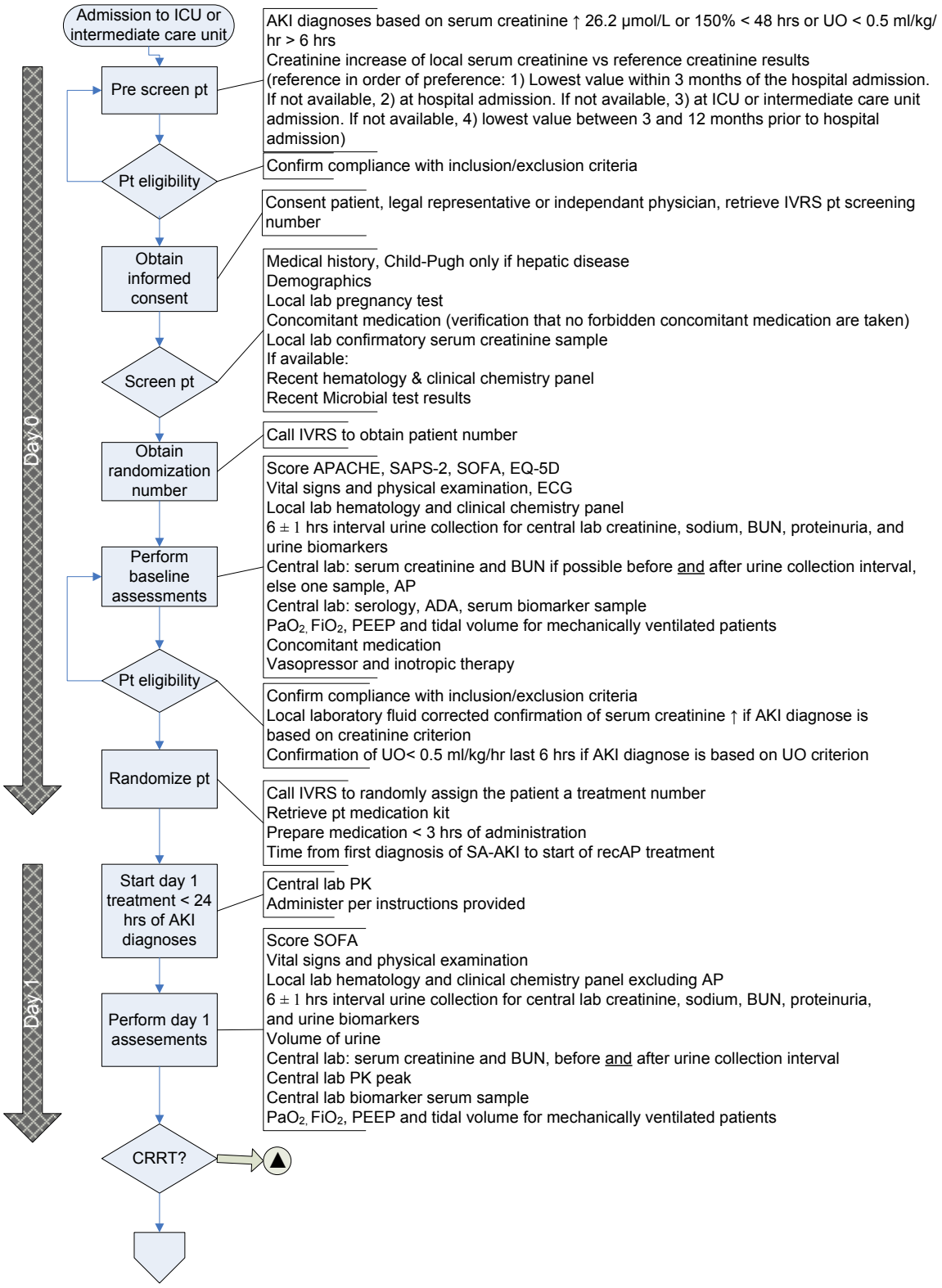
v. The actual stop date is collected for calculation of shock-free days. Only required when the patient is in the ICU or intermediate care unit.

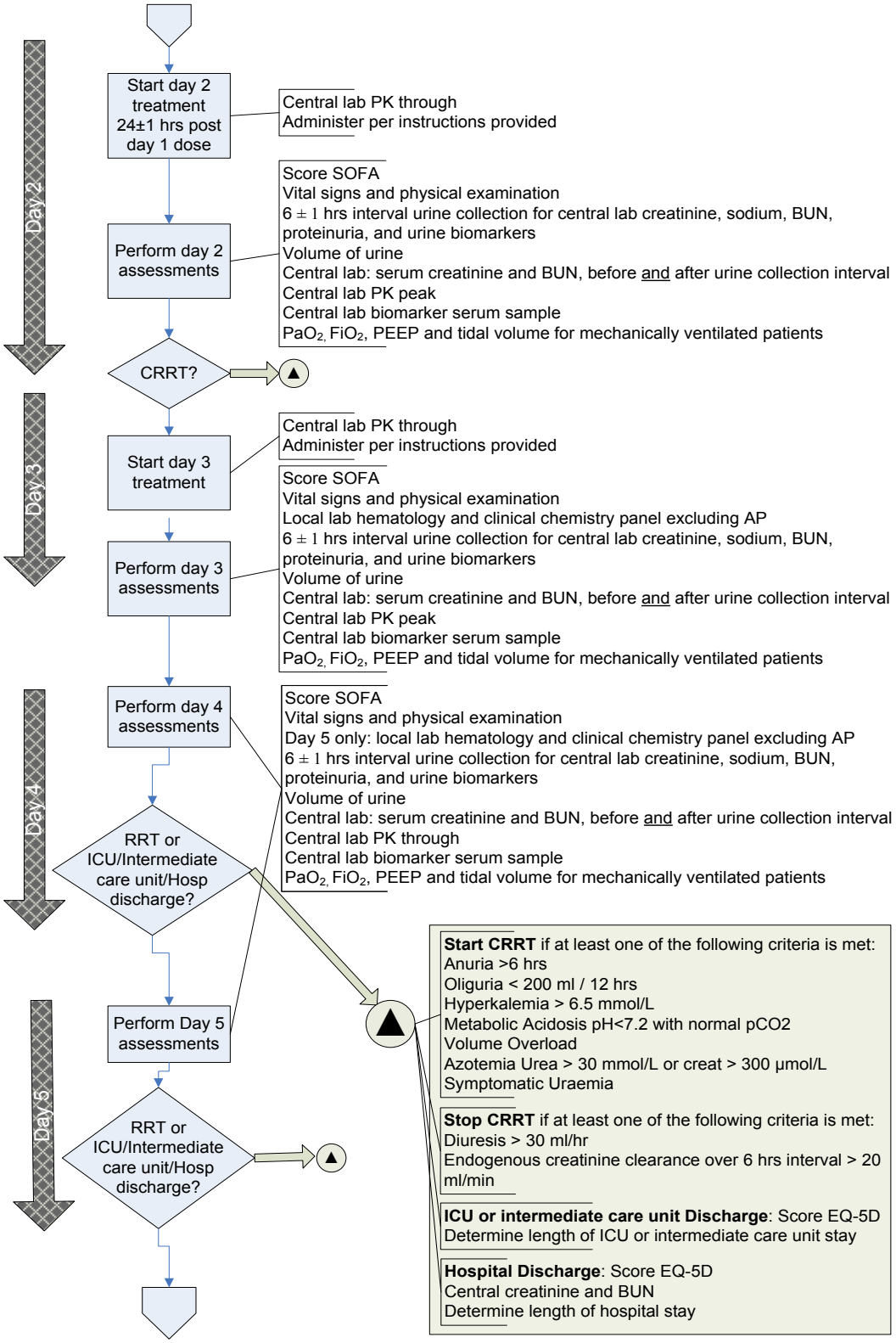
w. Daily, as long as the patient requires mechanical ventilation. As appropriate, record start and stop dates and times of mechanical ventilation, including the settings required and the O_2 in the blood.

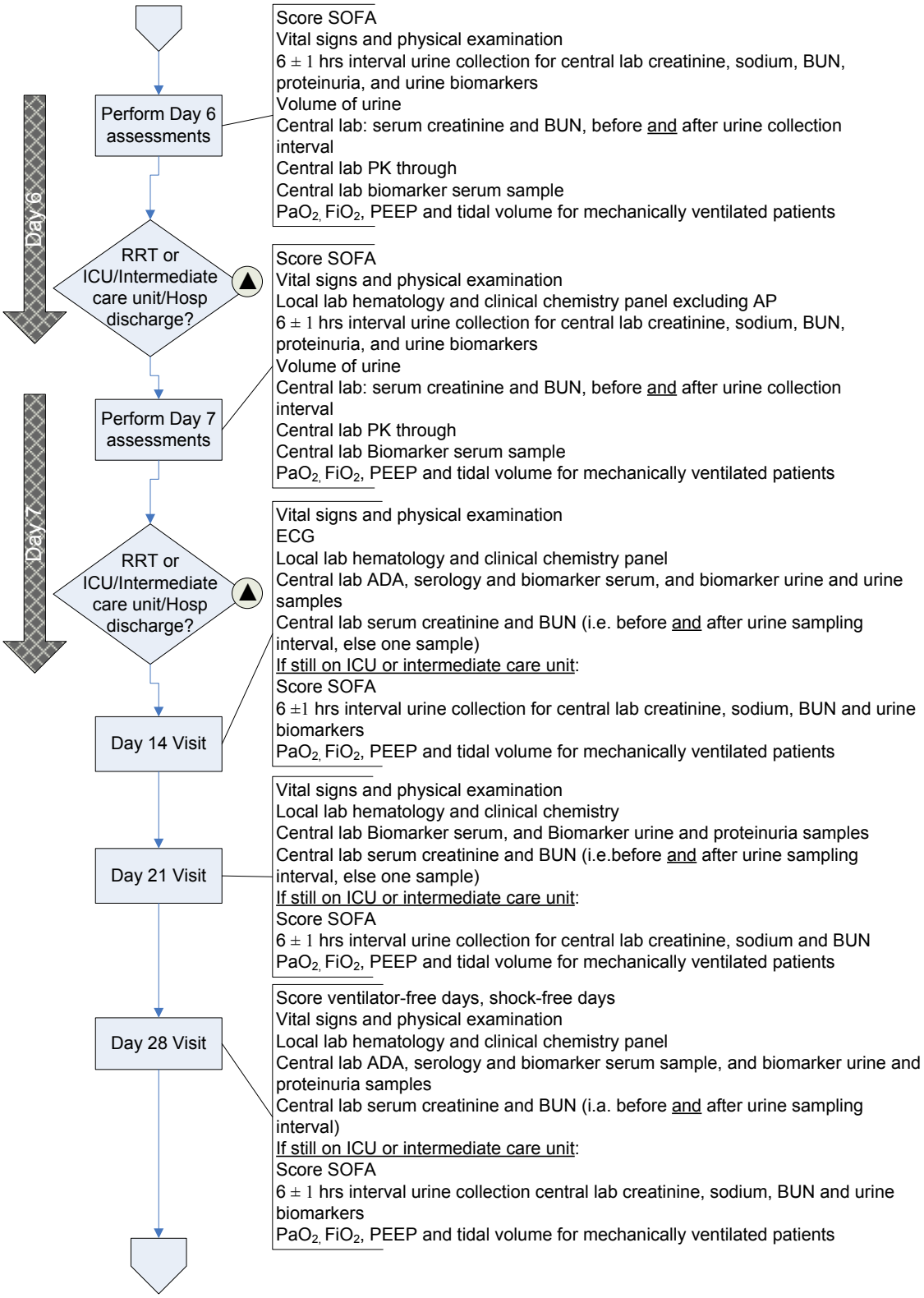
x. Actual admission dates and both planned and actual discharge dates from the ICU or intermediate care unit and hospital (planned meaning when decision is taken to discharge the patient, not necessarily being the same as the actual discharge date, e.g., because of lack of beds on the regular ward).

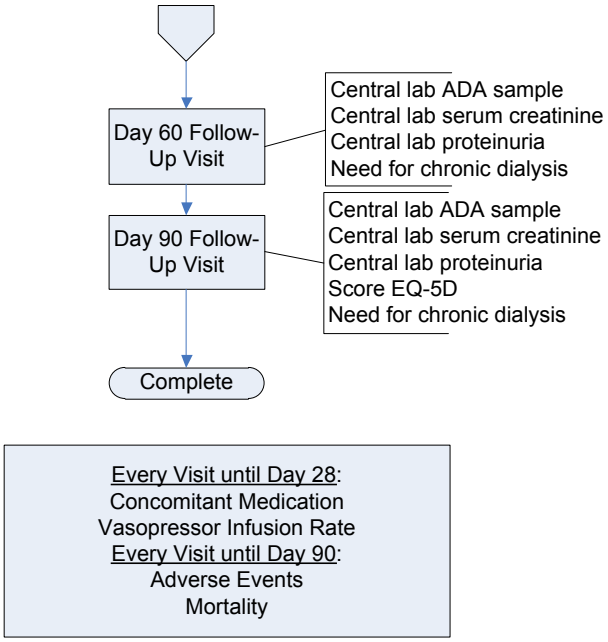
13.2 Appendix 2: Flowchart of Assessments

Figure 13-1 Flowchart of Assessments


Admission to ICU or intermediate care unit
Pre screen pt
Pt eligibility
Obtain informed consent
Screen pt
Obtain randomization number
Perform baseline assessments
Pt eligibility
Randomize pt
Start day 1 treatment < 24 hrs of AKI diagnoses
Perform day 1 assesements
CRRT?
Day 0
Day 1
AKI diagnoses based on serum creatinine ↑ 26.2 µmol/L or 150% < 48 hrs or UO < 0.5 ml/kg/ hr > 6 hrs
Creatinine increase of local serum creatinine vs reference creatinine results (reference in 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)
Confirm compliance with inclusion/exclusion criteria
Consent patient, legal representative or independant physician, retrieve IVRS pt screening number
Medical history, Child-Pugh only if hepatic disease
Demographics
Local lab pregnancy test
Concomitant medication (verification that no forbidden concomitant medication are taken)
Local lab confirmatory serum creatinine sample
If available:
Recent hematology & clinical chemistry panel
Recent Microbial test results
Call IVRS to obtain patient number
Score APACHE, SAPS-2, SOFA, EQ-5D
Vital signs and physical examination, ECG
Local lab hematology and clinical chemistry panel
6 ± 1 hrs interval urine collection for central lab creatinine, sodium, BUN, proteinuria, and urine biomarkers
Central lab: serum creatinine and BUN if possible before and after urine collection interval, else one sample, AP
Central lab: serology, ADA, serum biomarker sample
PaO2, FiO2, PEEP and tidal volume for mechanically ventilated patients
Concomitant medication
Vasopressor and inotropic therapy
Confirm compliance with inclusion/exclusion criteria
Local laboratory fluid corrected confirmation of serum creatinine ↑ if AKI diagnose is based on creatinine criterion
Confirmation of UO< 0.5 ml/kg/hr last 6 hrs if AKI diagnose is based on UO criterion
Call IVRS to randomly assign the patient a treatment number
Retrieve pt medication kit
Prepare medication < 3 hrs of administration
Time from first diagnosis of SA-AKI to start of recAP treatment
Central lab PK
Administer per instructions provided
Score SOFA
Vital signs and physical examination
Local lab hematology and clinical chemistry panel excluding AP
6 ± 1 hrs interval urine collection for central lab creatinine, sodium, BUN, proteinuria, and urine biomarkers
Volume of urine
Central lab: serum creatinine and BUN, before and after urine collection interval
Central lab PK peak
Central lab biomarker serum sample
PaO2, FiO2, PEEP and tidal volume for mechanically ventilated patients


Day 2
Start day 2 treatment 24±1 hrs post day 1 dose
Central lab PK through
Administer per instructions provided
Perform day 2 assessments
Score SOFA
Vital signs and physical examination
6 ± 1 hrs interval urine collection for central lab creatinine, sodium, BUN, proteinuria, and urine biomarkers
Volume of urine
Central lab: serum creatinine and BUN, before and after urine collection interval
Central lab PK peak
Central lab biomarker serum sample
PaO2, FiO2, PEEP and tidal volume for mechanically ventilated patients
CRRT?
Day 3
Start day 3 treatment
Central lab PK through
Administer per instructions provided
Perform day 3 assessments
Score SOFA
Vital signs and physical examination
Local lab hematology and clinical chemistry panel excluding AP
6 ± 1 hrs interval urine collection for central lab creatinine, sodium, BUN, proteinuria, and urine biomarkers
Volume of urine
Central lab: serum creatinine and BUN, before and after urine collection interval
Central lab PK peak
Central lab biomarker serum sample
PaO2, FiO2, PEEP and tidal volume for mechanically ventilated patients
Day 4
Perform day 4 assessments
Score SOFA
Vital signs and physical examination
Day 5 only: local lab hematology and clinical chemistry panel excluding AP
6 ± 1 hrs interval urine collection for central lab creatinine, sodium, BUN, proteinuria, and urine biomarkers
Volume of urine
Central lab: serum creatinine and BUN, before and after urine collection interval
Central lab PK through
Central lab biomarker serum sample
PaO2, FiO2, PEEP and tidal volume for mechanically ventilated patients
RRT or ICU/Intermediate care unit/Hosp discharge?
Perform Day 5 assessments
Day 5
RRT or ICU/Intermediate care unit/Hosp discharge?
Start CRRT if at least one of the following criteria is met:
Anuria >6 hrs
Oliguria < 200 ml / 12 hrs
Hyperkalemia > 6.5 mmol/L
Metabolic Acidosis pH<7.2 with normal pCO2
Volume Overload
Azotemia Urea > 30 mmol/L or creat > 300 µmol/L
Symptomatic Uraemia
Stop CRRT if at least one of the following criteria is met:
Diuresis > 30 ml/hr
Endogenous creatinine clearance over 6 hrs interval > 20 ml/min
ICU or intermediate care unit Discharge: Score EQ-5D
Determine length of ICU or intermediate care unit stay
Hospital Discharge: Score EQ-5D
Central creatinine and BUN
Determine length of hospital stay


Day 6
Perform Day 6 assessments
Score SOFA
Vital signs and physical examination
6 ± 1 hrs interval urine collection for central lab creatinine, sodium, BUN, proteinuria, and urine biomarkers
Volume of urine
Central lab: serum creatinine and BUN, before and after urine collection interval
Central lab PK through
Central lab biomarker serum sample
PaO2, FiO2, PEEP and tidal volume for mechanically ventilated patients
RRT or ICU/Intermediate care unit/Hosp discharge?
Day 7
Perform Day 7 assessments
Score SOFA
Vital signs and physical examination
Local lab hematology and clinical chemistry panel excluding AP
6 ± 1 hrs interval urine collection for central lab creatinine, sodium, BUN, proteinuria, and urine biomarkers
Volume of urine
Central lab: serum creatinine and BUN, before and after urine collection interval
Central lab PK through
Central lab Biomarker serum sample
PaO2, FiO2, PEEP and tidal volume for mechanically ventilated patients
RRT or ICU/Intermediate care unit/Hosp discharge?
Day 14 Visit
Vital signs and physical examination
ECG
Local lab hematology and clinical chemistry panel
Central lab ADA, serology and biomarker serum, and biomarker urine and urine samples
Central lab serum creatinine and BUN (i.e. before and after urine sampling interval, else one sample)
If still on ICU or intermediate care unit:
Score SOFA
6 ±1 hrs interval urine collection for central lab creatinine, sodium, BUN and urine biomarkers
PaO2, FiO2, PEEP and tidal volume for mechanically ventilated patients
Day 21 Visit
Vital signs and physical examination
Local lab hematology and clinical chemistry
Central lab Biomarker serum, and Biomarker urine and proteinuria samples
Central lab serum creatinine and BUN (i.e.before and after urine sampling interval, else one sample)
If still on ICU or intermediate care unit:
Score SOFA
6 ± 1 hrs interval urine collection for central lab creatinine, sodium and BUN
PaO2, FiO2, PEEP and tidal volume for mechanically ventilated patients
Day 28 Visit
Score ventilator-free days, shock-free days
Vital signs and physical examination
Local lab hematology and clinical chemistry panel
Central lab ADA, serology and biomarker serum sample, and biomarker urine and proteinuria samples
Central lab serum creatinine and BUN (i.a. before and after urine sampling interval)
If still on ICU or intermediate care unit:
Score SOFA
6 ± 1 hrs interval urine collection central lab creatinine, sodium, BUN and urine biomarkers
PaO2, FiO2, PEEP and tidal volume for mechanically ventilated patients



Abbreviations: ADA = anti-drug antibodies; AKI = acute kidney injury; AP = alkaline phosphatase; APACHE = acute physiology and chronic health evaluation; BUN = blood urea nitrogen; CRRT = continuous renal replacement therapy; ECG = electrocardiogram; FiO2 = fraction of inspired oxygen; Hosp = hospital; hrs = hours; ICU = intensive care unit; IVRS = interactive voice response system; lab = laboratory; PD = pharmacodynamics; PEEP = positive end expiratory pressure; PK = pharmacokinetics; pt = patient; recAP = recombinant human alkaline phosphatase; SAPS-2 = simplified acute physiology score 2; SOFA = sequential organ failure assessment; UO = urine output.

13.3 Appendix 3: Fluid-Corrected Serum Creatinine Assessment

Fluid-corrected serum creatinine will be assessed following the methodology provided by Macedo et al (2010):

Adjusted creatinine = serum creatinine × correction factor

Correction factor = (hospital admission weight [kg] × 0.6 + Σ [daily cumulative fluid balance (L)])/(hospital admission weight × 0.6).

Serum creatinine measures are not to be taken from the beginning of when a bolus is given until 30 minutes following completion, as the increased dilution during this period is not considered in the formulas provided (Pickering et al 2013).

References:

Macedo E, Bouchard J, Soroko SH, et al. Fluid accumulation, recognition and staging of acute kidney injury in critically-ill patients. Crit Care. 2010;14(3):R82.

Pickering JW, Ralib AM, Endre ZH. Combining creatinine and volume kinetics identifies missed cases of acute kidney injury following cardiac arrest. Crit Care. 2013;17(1):R7.

13.4 Appendix 4: Weight Ranges and Pre-calculated Corresponding Volumes

Table 13-2 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: IMP = investigational medical product.

13.5 Appendix 5: Iohexol and Exogenous Creatinine Clearance Protocol (Including Pharmacodynamics) for Selected Sites Only

13.5.1 Introduction

Accurate measurement of glomerular filtration rate (GFR) is valuable for evaluating kidney function and nephrotoxicity in a variety of clinical research and patient care settings. Accurate GFR determinations are recommended to appropriately assess the value of pharmacotherapeutic interventions aimed at improving tubular microvascular hemodynamics, inflammation and slowing the progression of renal disease.

The measure mostly used for the determination of the kidney function is the creatinine clearance as proxy for GFR. However, creatinine clearance is not fully representative in unstable situations, such as acute kidney injury, because it is influenced by various factors (e.g., diet, muscle mass, physical activity, and excretion and resorption in the kidney) and therefore results of creatinine clearance can be an over- or under-estimation. Creatinine clearance is routinely used to determine the function of the kidneys irrespective of the drawbacks, since it is inexpensive, non-invasive, and easy to perform.

Marker compounds such as iothalamate, iohexol, or inulin have been used extensively to measure GFR. Although renal clearance of these markers during continuous infusion is considered the gold standard, simplified methods using limited sampling after intravenous bolus administration with and without urine collection have been proposed.

The GFR estimation methods using creatinine concentration have been described as unreliable in some subgroups of the general population, especially in patients with non-steady state renal function. As iohexol has the main characteristics of a convenient marker of GFR, its plasma clearance seems to be the best choice to determine GFR in controlled laboratory settings. Contrary to determination of renal clearance of inulin, iohexol plasma clearance does not require continuous intravenous infusion.

It is generally assumed that endogenous creatinine clearance is not influenced by continuous renal replacement therapy. In order to reliably determine potential changes in endogenous creatinine clearance, any change in creatinine clearance needs to be excluded. 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.

13.5.2 Iohexol Dosing

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.

13.5.3 Iohexol Sampling

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. After centrifugation (500 × g, 10 minutes), the remaining approximately 1mL of plasma will be divided into 2 Micronic 1.4-mL cryotubes and stored at –80ºC, until batched shipments to the central laboratory are sent and further processing of one of each cryotubes at the Hillcrest Medical Center, United States, can be done.

Five-milliliter urine samples will be collected prior to the bolus infusions, and the urine bag will be emptied. Starting with freshly produced urine, 5-mL samples will be taken at 60, 120, and 360 minutes directly from the Foley catheter. An additional 5-mL sample will be taken from the combined total volume that was collected during the 6 ± 1 hour urine collection interval. The volumes of urine produced at the 60-, 120-, and 360-minute time points will be entered in the electronic case report form (eCRF). The total volume of urine collected in the 6 ± 1 hour interval will be corrected for the volume of samples taken during this interval and also entered in the eCRF. Urine tubes will be stored at –80ºC, until batched shipments to the central laboratory are sent and further processing of the urine at the Hillcrest Medical Center can be done.

The sample time points of effluent will be at t = 360 minutes of the urine collection interval, analogous to the time points for serum creatinine determinations, as needed for endogenous creatinine clearance calculations. Two samples of 1 mL will be taken from the effluent and stored in Micronic 1.4-mL cryotubes at –80ºC, until batched shipments to the central

laboratory are sent and further processing of one of each cryotube at the Hillcrest Medical Center can be done. The total volume of effluent will be recorded in the eCRF.

Although urine collection for 6 hours is preferred, a time window of ± 1 hour is allowed for pragmatic reasons adding flexibility to the schedule. In case the urine sampling interval is not exactly 6 hours, the last urine and blood sampling time points will have to match, e.g., with a 5-hour urine collection interval the last urine and blood sample will be obtained at t = 300 minutes.

13.5.4 Pharmacodynamic Assessments

A urine sample for pharmacodynamic assessment will be taken from the 6 ± 1 hour urine collection as required for calculation of the time-corrected endogenous creatinine clearance: pre-dose and post-dose on Day 1, post-dose on Day 2 and Day 3, and on Day 4, 5, 6, and 7. Urinary levels of adenosine triphosphate (ATP), adenosine diphosphate (ADP), adenosine monophosphate (AMP), cyclic AMP (cAMP), and adenosine will be analyzed centrally from this sample.

13.5.5 Statistical Data Analyses

13.5.5.1 Iohexol and Creatinine Clearance

Results from the measurement of iohexol and endogenous and exogenous creatinine clearance will be investigated in an exploratory manner with more details provided in the statistical analysis plan (SAP) (e.g., measured GFR, endogenous creatinine clearance in relation to exogenous creatinine clearance).

Iohexol clearance ($Cl_{Iohexol}$), as a gold standard measure of GFR, will be correlated to creatinine clearance at the different time points in a subset of patients.

The $Cl_{Iohexol}$ will be calculated by a 2-compartment model where $Cl_{Iohexol}$ = dose/area under the curve from 0 to infinity, with extrapolation from 6 hours to infinity, and potentially a more refined model.

Only descriptive methods will be applied for assessing exogenous creatinine clearance.

Effective renal plasma flow will be correlated with the iohexol clearances and creatinine clearances.

13.5.5.2 Pharmacodynamics

Endpoints for pharmacodynamics will be urinary levels of ATP, ADP, AMP, cAMP, and adenosine.

Urinary levels of ATP, ADP, AMP, cAMP, and adenosine will be analyzed in an exploratory fashion.

13.5.6 References

Castagnet S, Blasco H, Vourc'h P, et al. Routine determination of GFR in renal transplant recipients by HPLC quantification of plasma iohexol concentrations and comparison with estimated GFR. J Clin Lab Anal. 2012;26(5):376-83.

Laroute V, Lefebvre HP, Costes G, et al. Measurement of glomerular filtration rate and effective renal plasma flow in the conscious beagle dog by single intravenous bolus of iohexol and p-aminohippuric acid. J Pharmacol Toxicol Methods. 1999;41(1):17-25.

13.5.7 Schedule of Assessments for Iohexol and Exogenous Creatinine Clearance (Including Pharmacodynamics)

Table 13-3 Schedule of Assessments for Iohexol and Exogenous Creatinine Clearance

	Day 1					Day 2 to Day 6		Day 7 or ICU discharge				
	Just prior to bolus iohexol	**0 min**	**60 min**	**120 min**	**360 min**	**0 min**	**360 min**	**Just prior to bolus iohexol**	**0 min**	**60 min**	**120 min**	**360 min**
Iohexol bolus		X							X			
Urine sample from collection bag (5 mL)	X				X		X	X				X
6 ± 1 hour urine collection		→	→	→	X[a]	→	X		→	→	→	X[a]
Direct sample from Foley catheter (5 mL)			X	X	X					X	X	X
Blood sampling iohexol	X		X	X	X			X		X	X	X
Regular blood sampling creatinine /BUN[b]	X				X[a]	X	X	X				X[a]
RRT effluent sample[c]					X		X					X

Abbreviations: BUN = blood urea nitrogen; ICU = intensive care unit; min = minute; RRT = renal replacement therapy.

a. For any deviation of 6 ± 1 hour urine sampling, the regular blood sample for central laboratory creatinine/BUN assessment should be taken at the same deviating time point.

b. 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, 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 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 the approximately 6 hours will be entered in the electronic case report form.

[c.] When the patient is on RRT, a sample of the effluent of the RRT machine should be taken at the end of the time interval of the urine collection period.

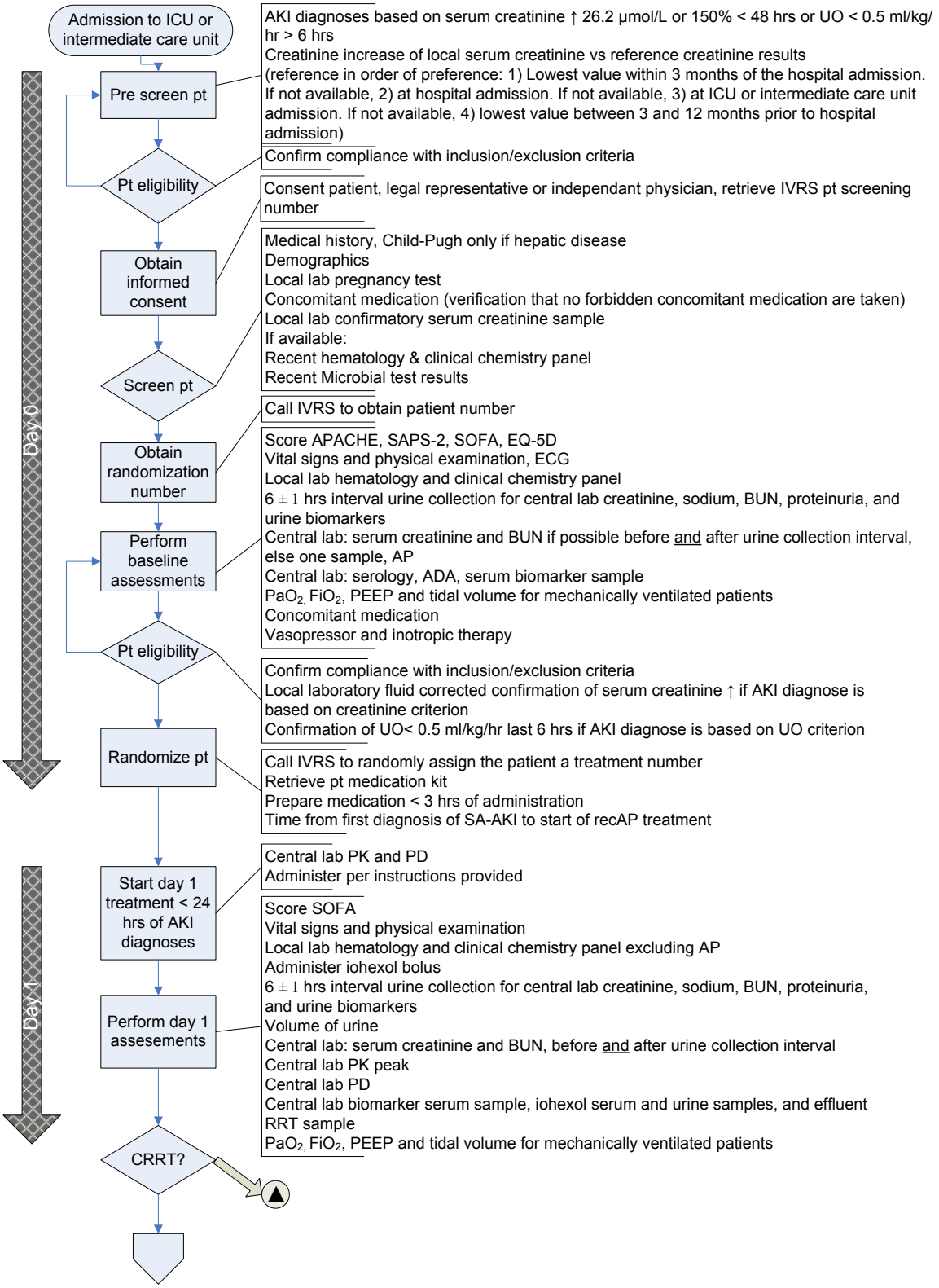
Table 13-4 Schedule of Assessments for Pharmacodynamics

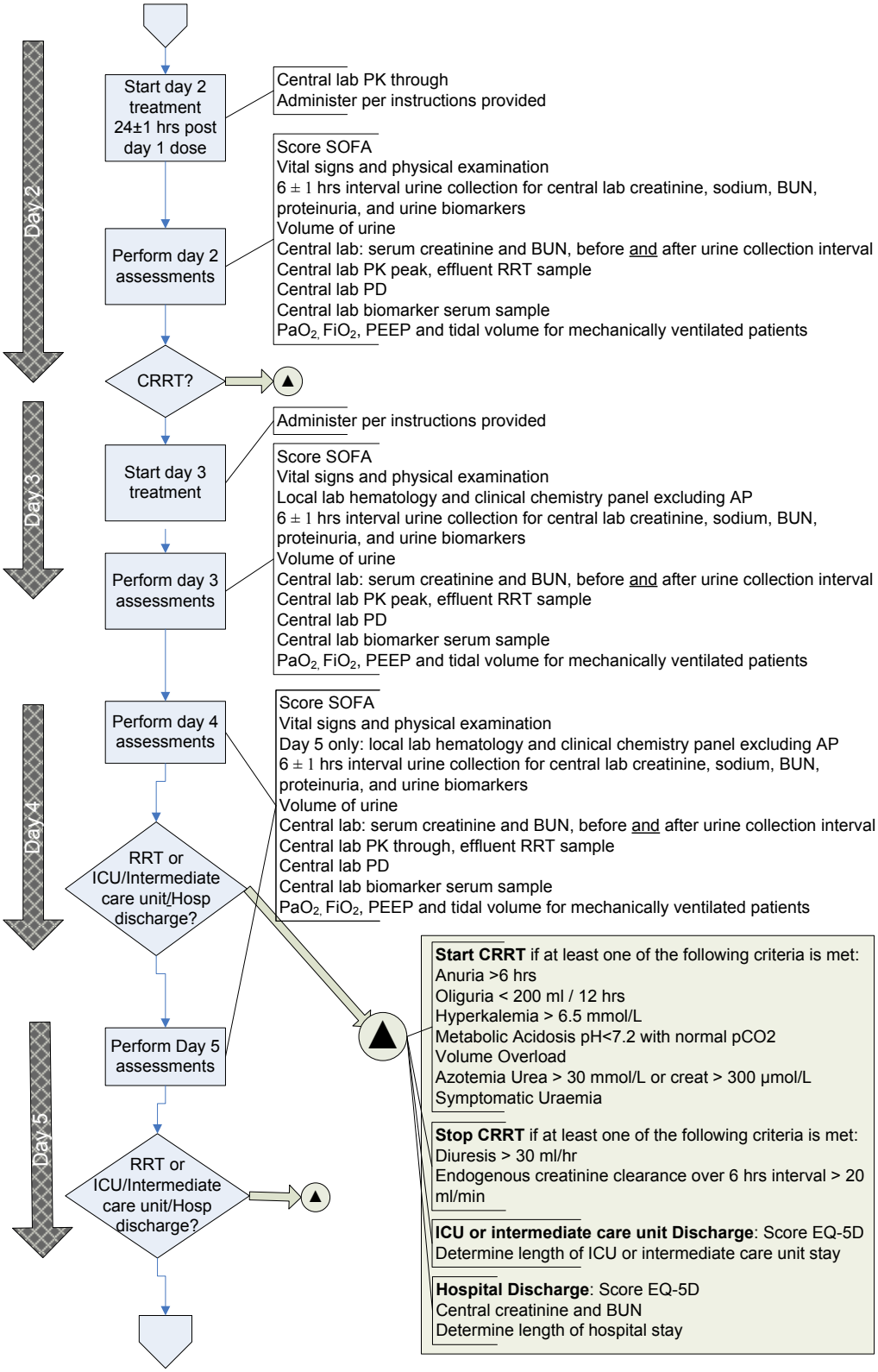
Assessment	**Screening (≤ 24 h)**	**Baseline (≤ 24 h)**	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7 (± 1 day)**	**Day 14 (± 2 days)**	**Day 21 (± 3 days)**	**Day 28 (± 3 days)**	**Day 60 (± 5 days)**	**Day 90 (± 10 days)**
Pharmacodynamics, central laboratory			X	X	X	X	X	X	X					

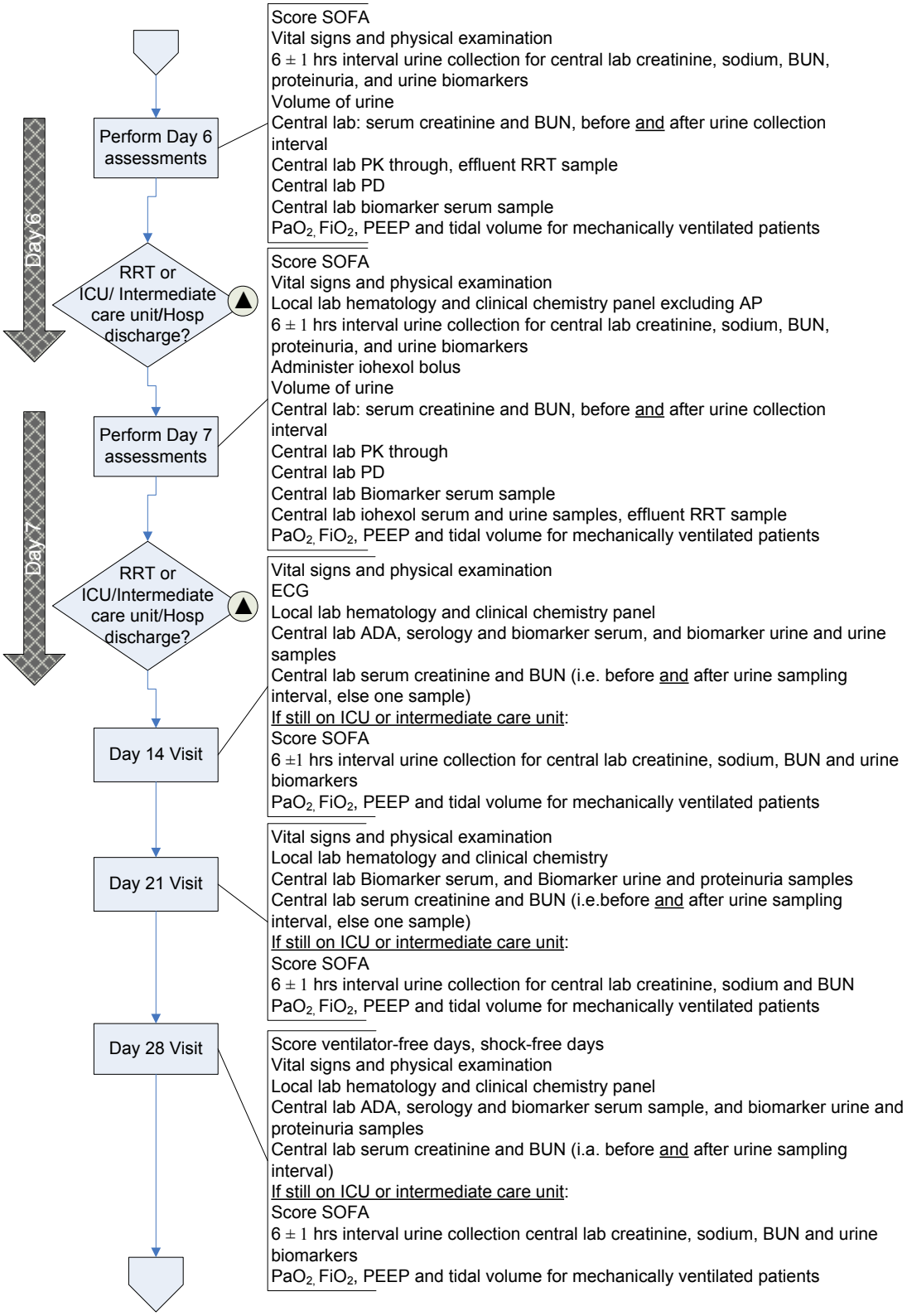
Abbreviation: h = hour.

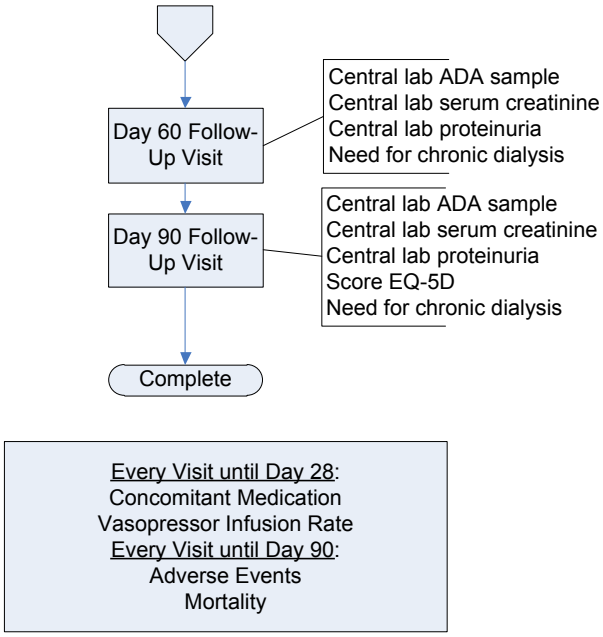
13.5.8 Flowchart of Assessments for Iohexol and Exogenous Creatinine Clearance (Including Pharmacodynamics)

Figure 13-2 Flowchart of Assessments for Iohexol and Exogenous Creatinine Clearance (Including Pharmacodynamics)


Admission to ICU or intermediate care unit
Pre screen pt
Pt eligibility
Obtain informed consent
Screen pt
Obtain randomization number
Perform baseline assessments
Pt eligibility
Randomize pt
Start day 1 treatment < 24 hrs of AKI diagnoses
Perform day 1 assesements
CRRT?
Day 0
Day 1
AKI diagnoses based on serum creatinine ↑ 26.2 µmol/L or 150% < 48 hrs or UO < 0.5 ml/kg/hr > 6 hrs
Creatinine increase of local serum creatinine vs reference creatinine results (reference in 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)
Confirm compliance with inclusion/exclusion criteria
Consent patient, legal representative or independant physician, retrieve IVRS pt screening number
Medical history, Child-Pugh only if hepatic disease
Demographics
Local lab pregnancy test
Concomitant medication (verification that no forbidden concomitant medication are taken)
Local lab confirmatory serum creatinine sample
If available:
Recent hematology & clinical chemistry panel
Recent Microbial test results
Call IVRS to obtain patient number
Score APACHE, SAPS-2, SOFA, EQ-5D
Vital signs and physical examination, ECG
Local lab hematology and clinical chemistry panel
6 ± 1 hrs interval urine collection for central lab creatinine, sodium, BUN, proteinuria, and urine biomarkers
Central lab: serum creatinine and BUN if possible before and after urine collection interval, else one sample, AP
Central lab: serology, ADA, serum biomarker sample
PaO2, FiO2, PEEP and tidal volume for mechanically ventilated patients
Concomitant medication
Vasopressor and inotropic therapy
Confirm compliance with inclusion/exclusion criteria
Local laboratory fluid corrected confirmation of serum creatinine ↑ if AKI diagnose is based on creatinine criterion
Confirmation of UO< 0.5 ml/kg/hr last 6 hrs if AKI diagnose is based on UO criterion
Call IVRS to randomly assign the patient a treatment number
Retrieve pt medication kit
Prepare medication < 3 hrs of administration
Time from first diagnosis of SA-AKI to start of recAP treatment
Central lab PK and PD
Administer per instructions provided
Score SOFA
Vital signs and physical examination
Local lab hematology and clinical chemistry panel excluding AP
Administer iohexol bolus
6 ± 1 hrs interval urine collection for central lab creatinine, sodium, BUN, proteinuria, and urine biomarkers
Volume of urine
Central lab: serum creatinine and BUN, before and after urine collection interval
Central lab PK peak
Central lab PD
Central lab biomarker serum sample, iohexol serum and urine samples, and effluent RRT sample
PaO2, FiO2, PEEP and tidal volume for mechanically ventilated patients


Day 2
Start day 2 treatment 24±1 hrs post day 1 dose
Central lab PK through
Administer per instructions provided
Perform day 2 assessments
Score SOFA
Vital signs and physical examination
6 ± 1 hrs interval urine collection for central lab creatinine, sodium, BUN, proteinuria, and urine biomarkers
Volume of urine
Central lab: serum creatinine and BUN, before and after urine collection interval
Central lab PK peak, effluent RRT sample
Central lab PD
Central lab biomarker serum sample
PaO2, FiO2, PEEP and tidal volume for mechanically ventilated patients
CRRT?
Day 3
Start day 3 treatment
Administer per instructions provided
Perform day 3 assessments
Score SOFA
Vital signs and physical examination
Local lab hematology and clinical chemistry panel excluding AP
6 ± 1 hrs interval urine collection for central lab creatinine, sodium, BUN, proteinuria, and urine biomarkers
Volume of urine
Central lab: serum creatinine and BUN, before and after urine collection interval
Central lab PK peak, effluent RRT sample
Central lab PD
Central lab biomarker serum sample
PaO2, FiO2, PEEP and tidal volume for mechanically ventilated patients
Day 4
Perform day 4 assessments
Score SOFA
Vital signs and physical examination
Day 5 only: local lab hematology and clinical chemistry panel excluding AP
6 ± 1 hrs interval urine collection for central lab creatinine, sodium, BUN, proteinuria, and urine biomarkers
Volume of urine
Central lab: serum creatinine and BUN, before and after urine collection interval
Central lab PK through, effluent RRT sample
Central lab PD
Central lab biomarker serum sample
PaO2, FiO2, PEEP and tidal volume for mechanically ventilated patients
RRT or ICU/Intermediate care unit/Hosp discharge?
Perform Day 5 assessments
Day 5
RRT or ICU/Intermediate care unit/Hosp discharge?
Start CRRT if at least one of the following criteria is met:
Anuria >6 hrs
Oliguria < 200 ml / 12 hrs
Hyperkalemia > 6.5 mmol/L
Metabolic Acidosis pH<7.2 with normal pCO2
Volume Overload
Azotemia Urea > 30 mmol/L or creat > 300 µmol/L
Symptomatic Uraemia
Stop CRRT if at least one of the following criteria is met:
Diuresis > 30 ml/hr
Endogenous creatinine clearance over 6 hrs interval > 20 ml/min
ICU or intermediate care unit Discharge: Score EQ-5D
Determine length of ICU or intermediate care unit stay
Hospital Discharge: Score EQ-5D
Central creatinine and BUN
Determine length of hospital stay


Day 6
Perform Day 6 assessments
Score SOFA
Vital signs and physical examination
6 ± 1 hrs interval urine collection for central lab creatinine, sodium, BUN, proteinuria, and urine biomarkers
Volume of urine
Central lab: serum creatinine and BUN, before and after urine collection interval
Central lab PK through, effluent RRT sample
Central lab PD
Central lab biomarker serum sample
PaO2, FiO2, PEEP and tidal volume for mechanically ventilated patients
RRT or ICU/ Intermediate care unit/Hosp discharge?
Score SOFA
Vital signs and physical examination
Local lab hematology and clinical chemistry panel excluding AP
6 ± 1 hrs interval urine collection for central lab creatinine, sodium, BUN, proteinuria, and urine biomarkers
Administer iohexol bolus
Volume of urine
Central lab: serum creatinine and BUN, before and after urine collection interval
Central lab PK through
Central lab PD
Central lab Biomarker serum sample
Central lab iohexol serum and urine samples, effluent RRT sample
PaO2, FiO2, PEEP and tidal volume for mechanically ventilated patients
Day 7
Perform Day 7 assessments
RRT or ICU/Intermediate care unit/Hosp discharge?
Vital signs and physical examination
ECG
Local lab hematology and clinical chemistry panel
Central lab ADA, serology and biomarker serum, and biomarker urine and urine samples
Central lab serum creatinine and BUN (i.e. before and after urine sampling interval, else one sample)
If still on ICU or intermediate care unit:
Score SOFA
6 ±1 hrs interval urine collection for central lab creatinine, sodium, BUN and urine biomarkers
PaO2, FiO2, PEEP and tidal volume for mechanically ventilated patients
Day 14 Visit
Vital signs and physical examination
Local lab hematology and clinical chemistry
Central lab Biomarker serum, and Biomarker urine and proteinuria samples
Central lab serum creatinine and BUN (i.e.before and after urine sampling interval, else one sample)
If still on ICU or intermediate care unit:
Score SOFA
6 ± 1 hrs interval urine collection for central lab creatinine, sodium and BUN
PaO2, FiO2, PEEP and tidal volume for mechanically ventilated patients
Day 21 Visit
Score ventilator-free days, shock-free days
Vital signs and physical examination
Local lab hematology and clinical chemistry panel
Central lab ADA, serology and biomarker serum sample, and biomarker urine and proteinuria samples
Central lab serum creatinine and BUN (i.a. before and after urine sampling interval)
If still on ICU or intermediate care unit:
Score SOFA
6 ± 1 hrs interval urine collection central lab creatinine, sodium, BUN and urine biomarkers
PaO2, FiO2, PEEP and tidal volume for mechanically ventilated patients
Day 28 Visit



Abbreviations: ADA = anti-drug antibodies; AKI = acute kidney injury; AP = alkaline phosphatase; APACHE = acute physiology and chronic health evaluation; BUN = blood urea nitrogen; CRRT = continuous renal replacement therapy; ECG = electrocardiogram; FiO2 = fraction of inspired oxygen; Hosp = hospital; hrs = hours; ICU = intensive care unit; IVRS = interactive voice response system; lab = laboratory; PEEP = positive end expiratory pressure; PK = pharmacokinetics; pt = patient; recAP = recombinant human alkaline phosphatase; RRT = renal replacement therapy; SAPS-2 = simplified acute physiology score 2; SOFA = sequential organ failure assessment; UO = urine output.

14 Protocol Amendments

The original Protocol Version 1.0 was dated 27 May 2014. Additions to the study protocol are shown in **bold** and deletions are shown in ~~strike through~~ text. Corrections of obvious typing errors or omissions and other editorial changes are not highlighted.

14.1 Amendment 1

The following changes from the original protocol were included in Protocol Version 2.0, including Amendment 1, dated 08 Oct 2014. In addition, some spelling mistakes and abbreviations have been corrected.

14.1.1 Change Number 1

The IND number was added to the protocol.

14.1.1.1 Reason:

To provide the IND number, which was obtained after Protocol Version 1.0 dated 27 May 2014 was finalized.

14.1.1.2 Protocol Section:

Cover page

Change to or Section Deleted:

IND number: ~~not available~~ **117605**

14.1.2 Change Number 2

Study design section has been amendment in regards to inclusion and exclusion criteria.

14.1.2.1 Reason:

To clarify that all inclusion criteria and none of the exclusion criteria are to be met.

14.1.2.2 Protocol Section:

Synopsis (Study Design)

Change to or Section Deleted:

As soon as possible when **all** inclusion and **none of the** 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 (Day 0), undergo baseline determinations, and start treatment with study drug (Day 1).

14.1.2.3 Protocol Section:

Section 3.1 Study Design

Change to or Section Deleted:

As soon as possible when **all** inclusion and **none of the** exclusion criteria are met (see Section 4.1), and after confirmation of continuing (i.e., not resolving) AKI by a fluid-corrected serum creatinine assessment (see Section 13.3) or urine output, eligible patients will be randomly assigned to a treatment group (Day 0), undergo baseline determinations, and start treatment with study drug (Day 1).

14.1.3 Change Number 3

Safety reporting requirements in Section 6.3 and Sections 6.3.14 to 6.5 (inclusive) have been amended in the study protocol and all exemptions to AE or SAE reporting have been removed. Consequently, investigators will be required to report all AEs, regardless of causality, occurring since the informed consent form has been signed until Day 30 after last dose of study drug or later for events possibly, probably, or definitely related to study drug.

14.1.3.1 Reason:

Per FDA request, all AEs and SAEs must be recorded in the eCRF.

14.1.3.2 Protocol Section:

Section 6.3 Study Procedures

Change to or Section Deleted:

Details of AE reporting ~~and exceptions~~ are provided in Section **6.3.14.3** ~~6.3.14.6~~.

14.1.3.3 Protocol Section:

Section 6.3.14 Adverse Events

Change to or Section Deleted:

The investigator is responsible for reporting all treatment-emergent AEs (TEAEs) that are observed or reported during the study, regardless of their relationship to study drug or their clinical significance. ~~However, because the patients participating in this study will experience clinical events that are related to severe sepsis, events leading to clinical outcomes related to severe sepsis are exempt from (S)AE reporting, unless the investigator considers the event(s) to have been caused by recAP. See Section 6.3.14.6 for details regarding events exempt from AE reporting~~.

14.1.3.4 Protocol Section:

Section 6.3.14.1 Definitions of Adverse Events

Change to or Section Deleted:

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 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.

~~However, in this study conducted in patients with severe sepsis, many medical events related to the underlying condition are expected to occur irrespective of treatment. Reporting all "events" will pose a burden on the investigators and staff and will likely not contribute to, and even complicate, the safety evaluation. Therefore, events leading to clinical outcomes that are related to severe sepsis are exempt from (S)AE reporting, as described in Section 6.3.14.6.~~

14.1.3.5 Protocol Section:

Section 6.3.14.2 Eliciting and Documenting Adverse Events

Change to or Section Deleted:

~~Treatment-emergent a~~**A**dverse events will be collected and assessed from the time the patient signs the ICF until ~~exit from the study or~~ 30 days after the last dose of the study drug~~, whichever is later~~ **or later for events possibly, probably, or definitely related to study drug.** Any AE that occurs during or after the first dose of study drug is considered treatment emergent.

…

In addition to patient observations, TEAEs will be documented from any data collected on the AE page of the eCRF (e.g., laboratory values, physical examination findings) or identified from review of other documents (e.g., patient diaries) that are relevant to patient safety.

~~Events leading to clinical outcomes related to severe sepsis are exempt from (S)AE recording in the eCRF, unless the investigator considers the event(s) to have been possibly, probably, or definitely caused by recAP. See Section 6.3.14.6 for details regarding events exempt from (S)AE reporting.~~

14.1.3.6 Protocol Section:

Section 6.3.14.3 Reporting Adverse Events

Change to or Section Deleted:

All ~~TE~~AEs reported or observed after the patient signs the ICF~~, except those described in Section 6.3.14.6,~~ will be recorded on the AE page of the eCRF.

…

Any AE that meets SAE criteria (see Section 6.3.14.1) must be reported to the PPD Pharmacovigilance Department immediately (i.e., within 24 hours) after the time site staff first learn about the event.~~, unless it is exempt from reporting as per Section 6.3.14.6.~~

14.1.3.7 Protocol Section:

Section 6.3.14.5 Assessment of Causality

Change to or Section Deleted:

The investigator's assessment of an AE's relationship to study drug is part of the documentation process~~, and only for events described in Section 6.3.14.6 will be a factor in determining what is or is not reported in the study~~. If there is any doubt as to whether a clinical observation is an AE or whether it is related to study drug, the event should be reported. ~~For events specified in Section 6.3.14.6, only those considered related to study drug (both AEs and SAEs) are required to be reported.~~

14.1.3.8 Protocol Section:

Section 6.3.14.6 Exemptions from Adverse Event Reporting

Change to or Section Deleted:

Entire section deleted.

14.1.3.9 Protocol Section:

Section 6.3.14.7 Follow-up of Patients Reporting Adverse Events

Change to or Section Deleted:

Renumbered to Section 6.3.14.6.

All TEAEs~~, except those exempt from reporting as per Section 6.3.14.6,~~ must be reported in detail on the appropriate page of the eCRF and followed to satisfactory resolution, until the investigator deems the event to be chronic or not clinically significant, or until the patient is considered to be stable.

14.1.3.10 Protocol Section:

Section 6.5 Laboratory Analyses

Change to or Section Deleted:

~~Any clinically significant safety and laboratory assessments that are associated with the underlying disease, i.e., severe sepsis, are not to be reported as (S)AEs, unless judged by the~~

~~investigator to be more severe than expected for the patient's condition or considered possibly, probably, or definitely related to study drug (see Section 6.3.14.6).~~

Any abnormal laboratory test results (hematology, clinical chemistry, or urinalysis) or other safety assessments (e.g., ECGs, vital sign measurements), including those that worsen from baseline, felt to be clinically significant in the medical and scientific judgment of the investigator, are to be recorded as (S)AEs~~, except those exempt from reporting as per Section 6.3.14.6.~~

14.1.3.11 Protocol Section:

Section 13.1 Appendix 1: Schedule of Assessments; Table 13-1 Schedule of Assessments

Change to or Section Deleted:

Deleted footnote:

~~x See Section 6.3.14.6 for exemptions.~~

14.1.4 Change Number 4

The following changes to vital sign measurements were implemented: 1) Vital sign monitoring requirements were amended to include at least hourly monitoring up to 6 hours after the start of infusion of IMP on Day 1; 2) Oxygen saturation was added to the vital sign assessments; 3) It was specified that blood pressure will be monitored non-invasively, or invasively via arterial line in patients who already have an arterial line placed as part of standard or care.

14.1.4.1 Reason:

Because postural hypotension was reported 3 hours after dosing of recAP in the Phase 1 study, monitoring of vital signs has been extended until 6 hours after the start of the study infusion on Day 1. Additionally, oxygen saturation has been added to the vital sign assessments because capturing measurements of this parameter during infusion and afterwards would not be associated with any risk to patients, while it would provide useful data on the safety of recAP infusion in a critically ill population, assist causality assessment for any associate drug reactions (especially cardiovascular and respiratory) that occur in close proximity to infusion, and help exclude hypoxia as cause of dizziness (which was observed

in the Phase 1 study but which mechanism is unknown). If the study demonstrated no effect on oxygen saturation of IMP infusion, this would provide additional support for safety of use of recAP in the critically ill patient population. Finally, text has been amended to clarify that blood pressure will be monitored non-invasively, or invasively via arterial line in patients who already have an arterial line placed as part of standard or care, as it is expected that a significant proportion of patients in this study will undergo invasive blood pressure monitoring via arterial line; thus, these readings should be acceptable.

14.1.4.2 Protocol Section:

Synopsis (Safety Assessments)

Change to or Section Deleted

The following assessments will be considered as safety measurements:

- Vital signs, including blood pressure, heart rate, **oxygen saturation,** respiratory rate, and body temperature at baseline and at all visits from Day 1 to Day 28. During the 3 dosing days (Day 1 to Day 3), repeated vital sign measurements will be performed.

14.1.4.3 Protocol Section:

Section 6.2.2 Baseline Assessments

Change to or Section Deleted

The following assessments will be performed at baseline after randomization (i.e., at study inclusion, not necessarily at ICU admission date):

- Vital signs (temperature, heart rate, respiratory rate, **oxygen saturation,** and blood pressure)

14.1.4.4 Protocol Section:

Section 6.2.4 Safety Assessments

Change to or Section Deleted:

The following assessments will be considered as safety measurements:

• Vital signs, including blood pressure, heart rate, **oxygen saturation,** respiratory rate, and body temperature at baseline and at all visits from Day 1 to Day 28. During the 3 dosing days (Day 1 to Day 3), repeated vital sign measurements will be performed (see Section 6.3.4).

14.1.4.5 Protocol Section:

Section 6.3.4 Vital Signs

Change to or Section Deleted:

Vital signs, including temperature, heart rate, **oxygen saturation,** respiratory rate and blood pressure, will be measured at baseline, and at all subsequent visits from Day 1 to Day 28 (see Section 13.1 [Table 13-1] and Section 13.2 [Figure 13-1]). Vital signs in ambulant patients will be measured 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.**

On visit days when study drug is administered (Days 1, 2 and 3), vital signs (excluding temperature) will be measured **and recorded** as follows:

Immediately before the administration of the study drug

Within 5 minutes of the start of the study drug infusion

30 minutes after the start of the study drug infusion

Immediately after the completion of the administration of the study drug, which includes post-dose saline flushing

~~Approximately~~ 30 and 60 minutes after completion of study drug administration.

Additionally, on Day 1 only, vital signs will be measured and recorded as follows:

2 hours, 3 hours, 4 hours, and 5 hours after the completion of study drug administration

14.1.4.6 Protocol Section:

Section 13.1 Appendix 1: Schedule of Assessments; Table 13 1 Schedule of Assessments

Change to or Section Deleted:

Vital signs (BP, HR, **OS**, RR, T)[g]

…

Footnotes:

Abbreviations: … **OS = oxygen saturation**

[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) ~~approximately~~ 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).**

14.1.5 Change Number 5

Accumulating safety and efficacy data during Part 1 of the study will be reviewed periodically and ad hoc by the DMC as described in the DMC charter, in addition to the already mentioned DMC role of selecting dose.

14.1.5.1 Reason:

Regular interim analyses of the accumulating safety data should be performed to enhance the safety of trial participants.

14.1.5.2 Protocol Section:

Section 10.1.1 External Data Monitoring Committee

Change to or Section Deleted:

An independent DMC will review the results of the interim analysis and select the optimum recAP dose for Part 2 of the study (see Section 7.7.5). **Accumulating safety and efficacy data will be reviewed at predefined milestones during Part 1 and Part 2 of the study by the independent DMC as described in the DMC charter.**

14.1.6 Change Number 6

The definition of the primary endpoint was amended from creatinine clearance to area under the time-corrected endogenous creatinine clearance curve from Day 1 to Day 7 (AUC_{1-7}). The formula for calculation of the AUC_{1-7} was amended to indicate that this will be the average of the time-corrected endogenous creatinine clearance values over the 7 days.

14.1.6.1 Reason:

Clarification required regarding the specific measurement to be used in the primary analysis and its calculation

14.1.6.2 Protocol Section:

Synopsis (Rationale, Efficacy Assessments, Sample Size, and Statistical Methods)

Change to or Section Deleted:

The primary endpoint, **area under the time-corrected endogenous** creatinine clearance **curve**, was chosen because…

…

Urine and serum creatinine determinations will be performed to enable calculation of time-corrected endogenous creatinine clearance~~, considered as the primary endpoint,~~ at all visits from Day 0 to Day 7, and calculated by the central laboratory. **The measurements from Day 1 to Day 7, inclusive, will be used to calculate the primary endpoint.** Only if reliable urine collection is possible, urine will be collected on Days 14, 21, and 28 for **time-corrected** endogenous creatinine clearance (i.e., patient is in the ICU or hospital).

…

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}])…

…

The primary efficacy endpoint, area under the **time-corrected endogenous** creatinine clearance curve from Day 1 to Day 7 (AUC_{1-7}), will be summarized using descriptive statistics and analyzed using analysis of variance with treatment and site as explanatory variables.

14.1.6.3 Protocol Section:

List of Abbreviations

Change to or Section Deleted:

AUC_{1-7}	area under the **time-corrected endogenous** creatinine clearance curve from Day 1 to Day 7

14.1.6.4 Protocol Section:

Section 3.1.1 Rationale of Study Design

Change to or Section Deleted:

Assuming comparable safety profiles, the most effective dose will be selected at the interim analysis by an independent DMC. The primary endpoint, **area under the time-corrected endogenous** creatinine clearance **curve**, 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.

14.1.6.5 Protocol Section:

Section 6.2.3 Efficacy Assessments

Change to or Section Deleted:

Urine and serum creatinine determinations will be performed to enable calculation of time-corrected endogenous creatinine clearance~~, considered as the primary endpoint,~~ at all

visits from Day 0 to Day 7, and calculated by the central laboratory. **The measurements from Day 1 to Day 7, inclusive, will be used to calculate the primary endpoint.** Only if reliable urine collection is possible, urine will be collected on Days 14, 21, and 28 for **time-corrected** endogenous creatinine clearance (i.e., patient is in the ICU or hospital). If reliable urine collection is not possible, serum creatinine samples still need to be taken for eGFR at all visits.

14.1.6.6 Protocol Section:

Section 6.3.8.1 Endogenous Creatinine and Blood Urea Nitrogen Clearance

Change to or Section Deleted:

The efficacy of the treatment will be investigated by calculating the time-~~adjusted~~ **corrected** endogenous creatinine clearance. Blood urea nitrogen clearance will also be measured. To calculate the **time-corrected endogenous** creatinine clearance, timed urine (including measurement of volume) and blood samples need to be collected.

Urine and serum creatinine determinations will be performed to enable calculation of time-corrected endogenous creatinine clearance~~, considered as the primary endpoint,~~ at all visits from Day 0 to Day 7, and calculated by the central laboratory. **The measurements from Day 1 to Day 7, inclusive, will be used to calculate the primary endpoint.** Only if reliable urine collection is possible, urine will be collected on Days 14, 21, and 28 for **time-corrected** endogenous creatinine clearance (i.e., patient is in the ICU or hospital).

14.1.6.7 Protocol Section:

Section 6.3.8.6 Pharmacodynamic Assessments

Change to or Section Deleted:

For a limited number of sites, a urine sample for ~~pharmacodynamic (~~PD~~)~~ assessment will be taken from the 6 ± 1 hour urine collection as required for calculation of the **time-corrected** endogenous creatinine clearance. Detailed instructions to these sites for PD assessments are provided in Section 13.5.

14.1.6.8 Protocol Section:

Section 7.1 Primary Endpoint

Change to or Section Deleted:

The primary endpoint **will be calculated from** ~~is~~ time-~~adjusted~~ **corrected** endogenous creatinine clearance **measurements on Day 1 (first measurement after treatment) to Day 7, inclusive. Time-corrected 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. The primary endpoint is the area under the time-corrected endogenous creatinine clearance curve from Day 1 to Day 7** ($AUC_{1\text{-}7}$)**,** ~~i.e., area under the creatinine clearance curve from Day 1 [first measurement after treatment] to Day 7, inclusive), with the creatinine clearance curve being the mean creatinine clearance on each day. $AUC_{1\text{-}7}$ is~~ calculated as ~~a weighted~~ **the** average of the **time-corrected endogenous** creatinine clearance values over the 7 days. **Specifically, d**~~D~~enoting C_i as the mean **time-corrected endogenous** creatinine clearance on Day*i*, $AUC_{1\text{-}7}$ is defined as:

$$AUC_{1-7} = \frac{1}{7}\sum_{i=1}^{7} C_i \quad \sout{AUC_{1-7} = \sum_{i=1}^{6} \frac{C_i + C_{i+1}}{2}}$$

…

14.1.6.9 Protocol Section:

Section 7.7.1 Analysis of Primary Efficacy Endpoint

Change to or Section Deleted:

The primary efficacy endpoint is **the area under the time-corrected** ~~adjusted~~ endogenous creatinine clearance **curve** from Day 1 to Day 7, i.e., ~~the~~ $AUC_{1\text{-}7}$.

Area under the **time-corrected endogenous** creatinine clearance curve from Day 1 to Day 7 will be summarized using descriptive statistics and analyzed using an analysis of variance (ANOVA) with treatment and site as explanatory variables.

14.1.6.10 Protocol Section:

Section 13.5.4 Pharmacodynamic Assessments

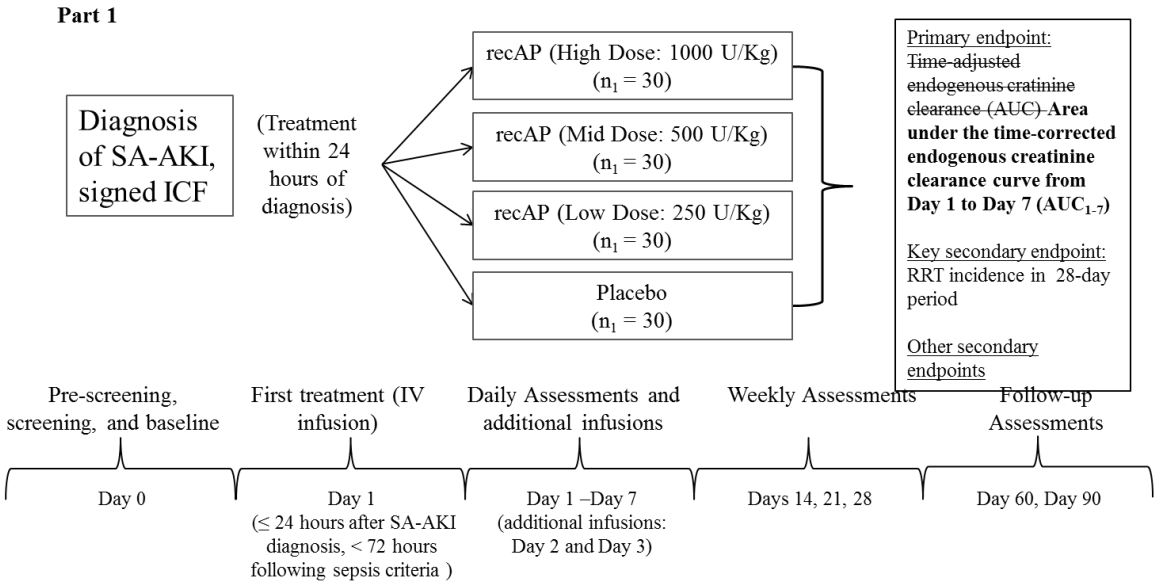
Change to or Section Deleted:

A urine sample for pharmacodynamic assessment will be taken from the 6 ± 1 hour urine collection as required for calculation of the **time-corrected** endogenous creatinine clearance…

14.1.6.11 Protocol Section:

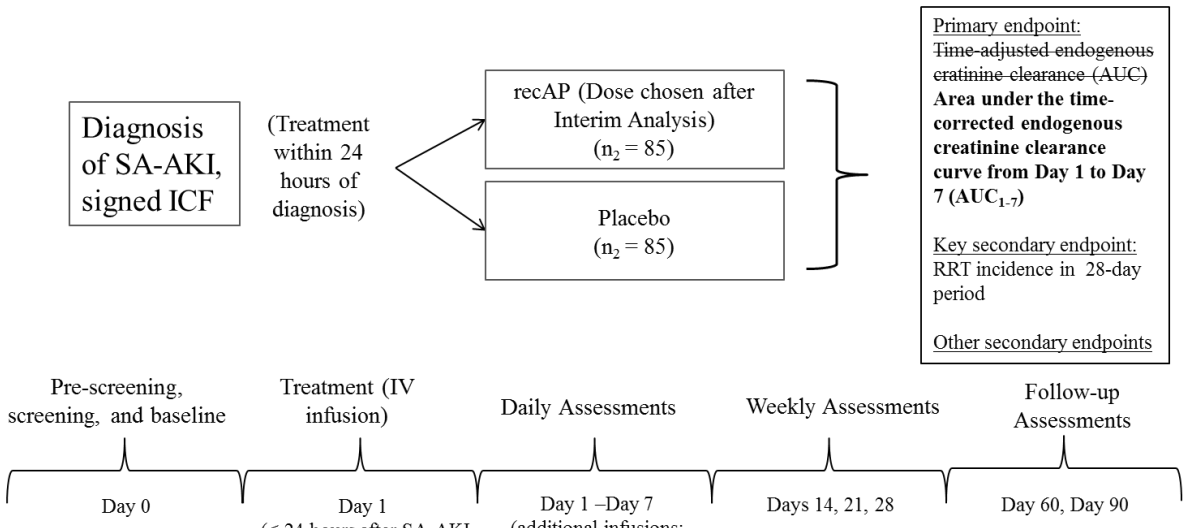
Figure 3-1 Study Design

Change to or Section Deleted:



Interim analysis to determine optimal active dose to compare with placebo in Part 2

Part 2



Abbreviations: ~~AUC = area under the concentration time curve;~~ ICF = informed consent form; ICU = intensive care unit; IV = intravenous; recAP = recombinant human alkaline phosphatase; RRT = renal replacement therapy; SA-AKI = sepsis-associated acute kidney injury.

14.1.7 Change Number 7

The following changes were made to the statistical methods: 1) The p_1 in the combination test is now specified as follows: for the single hypothesis, p_1 is the unadjusted p-value to compare optimal dose with placebo in Part 1, while for the intersection hypotheses, p_1 is the Dunnett-adjusted p-value in Part 1; 2) Any missing C_i values will be handled by linear interpolation where possible, otherwise they will be imputed by last observation carried forward or imputed as the baseline measurement from Day 0 (when there are no preceding post-baseline measurements to use); 3) n_1 and n_2 in the formula for the combination test are specified as the sample size per group in Parts 1 and 2 respectively.

14.1.7.1 Reason:

This is a proof-of-concept trial where strong control of type I error rate is not necessary because it is purely for learning. A learning trial provides an opportunity for fully exploring dose response from which a dose or doses can be selected with good precision for study in Phase 3 trials. Clarification is required to specify where the p_1 in the combination test should correspond to the Dunnett-adjusted p-value in Part 1. In addition, there should be baseline measurements available for all patients, which should be used for the imputation of C_i when such cases arise. Clarification is also needed to specify that n_1 and n_2 in the formula for the combination test represent the sample size per group in Parts 1 and 2 respectively, rather than the overall sample size in Part 1 or 2.

14.1.7.2 Protocol Section:

Synopsis (Statistical Methods)

Change or Section Deleted:

The analysis will be performed separately for Parts 1 and 2: for Part 1, $AUC_{1\text{-}7}$ will be compared between the ~~4 treatment groups using Dunnett test and a closed testing procedure~~ **3 recAP doses and 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 level α if it and all intersection hypotheses involving it are all rejected at local level α. The testing strategy used to combine results from Parts 1 and 2 will be ~~The results from Parts 1 and 2 will be combined using~~ a combination test based on the inverse normal method.

14.1.7.3 Protocol Section:

Section 7.1 Primary Endpoint

Change to or Section Deleted:

Any missing C_i values will be handled by linear interpolation ~~within the AUC calculation~~ where possible, otherwise they will be imputed by last observation carried forward (LOCF)**.** ~~or imputed as zero (~~**W**~~w~~hen there are no preceding **post-baseline** measurements to ~~carry forward).~~**use, the baseline measurement from Day 0 (i.e., prior to treatment) will be carried forward.**

14.1.7.4 Protocol Section:

Section 7.7.1 Analysis of Primary Efficacy Endpoint

Change to or Section Deleted:

The analysis will **be** performed separately for Parts 1 and 2: for Part 1, $AUC_{1\text{-}7}$ will be compared between the ~~4~~ **3 recAP doses and placebo.** ~~treatment groups using Dunnett test and a closed testing procedure.~~ This analysis will be considered in conjunction with the safety data to determine the optimal recAP dose for use in Part 2. For Part 2, the optimal recAP dose will be compared with 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 level α if it and all intersection hypotheses involving it are all rejected at local level α. The testing strategy used to combine results from Parts 1 and 2 will be** ~~The results from Parts 1 and 2 will be combined using~~ 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 Parts 1 and 2, respectively, $n = n_1 + n_2$, **Φ refers to the standard normal distribution, and p_1 and p_2 are the p-values from Parts 1 and 2, respectively. For the single hypothesis, p_1 is the unadjusted p-value to compare optimal dose with placebo in Part 1. For the intersection hypotheses, p_1 is the Dunnett-adjusted p-value in Part 1, and p_2 is the unadjusted p-value to compare optimal dose with placebo in Part 2 for all hypotheses.** ~~and p_1 and p_2 are the unadjusted p-values from Parts 1 and 2, respectively, and Φ refers to the standard normal distribution.~~

14.1.8 Change Number 8

Updates were made to the criteria leading to withdrawal from the study and discontinuation of study drug, and updates were made to the procedures followed after a patient withdraws or is discontinued from the study.

14.1.8.1 Reason:

To clarify that withdrawal from the study is not equivalent to discontinuation of study drug, and to clarify the procedures to follow when a withdrawal/discontinuation criterion is met.

14.1.8.2 Protocol Section:

Section 4.2.1 Reasons for Withdrawal/Discontinuation

Change to or Section Deleted:

Patients may withdraw from the study at any time and for any reason without prejudice to their future medical care by the investigator or at the study site. ~~Every effort should be made to keep patients in the study. The reasons for patients not completing the study will be recorded~~. **Investigators should attempt to determine the cause of withdrawal and, if desired by the patient, to let the patient return for the Day 90 visit (last safety only follow-up visit). The extent of a patient's withdrawal from the study (i.e., withdrawal from further study treatment, withdrawal from active participation in the study, withdrawal from any further contact) should be documented. Every effort should be**

taken to follow all randomized patients, to the extent that the patient will allow, for the full follow-up period.

~~A patient may be withdrawn from the study~~ **Treatment with study drug will be stopped** for any of the following reasons:

1. Any clinical adverse event (AE), laboratory abnormality, or intercurrent illness which, in the opinion of the investigator, indicates that continued ~~participation in the study~~ **treatment with study drug** is not in the best interest of the patient.
2. ~~Protocol non-compliance or violations.~~ **The patient withdraws consent or the investigator or sponsor decides to discontinue the patient's participation in the study.**
3 ~~The patient withdraws consent or the investigator or sponsor decides to discontinue the patient's participation in the study.~~ **AM-Pharma B.V. terminates the study.**

~~4. AM-Pharma B.V. terminates the study.~~

Additionally, treatment with study drug may be stopped in case of protocol non-compliance or violations. In this case, the investigator will contact the Medical Monitor to discuss risk/benefit balance of continued treatment with study drug.

In case study drug is discontinued early, the patient will continue follow-up in the study as per protocol to allow for intent-to-treat (ITT) analysis. If interrupted for any reason, re-starting of study drug should be discussed with the Medical Monitor.

The reason for and date of study drug discontinuation and the reason for and date of withdrawal from the study must be recorded in the electronic case report form (eCRF). If study drug is discontinued because of an AE or a clinically significant abnormal laboratory test result, evaluations will continue until the event has resolved or stabilized or until a determination of a cause unrelated to the study drug or study procedure is made. The specific event or laboratory finding(s) must be documented.

14.1.9 Change Number 9

It was clarified that patients who discontinue study drug or withdraw from the study will complete the end-of-study visit and, if desired by the patient, the follow-up assessments as per the study schedule (Day 60 and Day 90).

14.1.9.1 Reason:

It is critical to have complete and unbiased follow-up for long-term safety assessments.

14.1.9.2 Protocol Section:

Section 4.2.2 Handling of Withdrawals

Change to or Section Deleted:

Enrolled patients who discontinue study drug or active participation in the study will no longer receive study drug. When a patient withdraws from the study, the investigator will record the reason(s) for withdrawal on the relevant page of the eCRF. All patients who discontinue study drug or withdraw from the study prematurely will undergo all end-of-study (Day 28) assessments **immediately upon discontinuation. Investigators should attempt to determine the cause of withdrawal and, if desired by the patient, to let the patient return for the Day 90 visit (last safety only follow-up visit).** (~~including follow-up of ongoing serious AEs [SAEs]).~~ Patients who fail to return for final assessments will be contacted by the site in an attempt to have them comply with the protocol; attempts to contact patients will be recorded in the eCRF.

It is vital to obtain follow-up **safety** data on any patient withdrawn because of an AE or serious adverse event (SAE). In every case, efforts must be made to undertake protocol-specified, safety, follow-up procedures.

The reason for withdrawal of a patient must be recorded in the eCRF, if it can be ascertained. Any further treatment will be at the investigator's discretion and should be recorded. Every effort must be made to follow-up the patient and obtain information on ~~clinical outcome and~~ AEs. All data from withdrawals will be included in the final ITT analyses.

14.1.10 Change Number 10

The individual benefit for the patients was specified.

14.1.10.1 Reason:

According to *§ 43a paragraph 1number 4 AMG* (Austrian drug law), the benefit for the patient must be higher than the risk. The individual benefit is to be given.

14.1.10.2 Protocol Section:

New Section 3.1.3 Individual Benefit/Risk Considerations

Change to or Section Deleted:

Benefits for patients with sepsis and AKI are anticipated based on pharmacodynamic (PD) data and data from 2 Phase 2 studies described in the investigator's brochure. In the APSEP 02-01 study, BiAP was associated with a non-significant survival benefit versus placebo and clinically important benefits on renal function parameters of serum creatinine, inflammatory and kidney damage biomarkers and dialysis requirements. In the APREN01-01 study, BiAP significantly improved kidney function (determined by a composite endpoint of creatinine clearance, requirement for RRT and duration of RRT) and significantly reduced the length of ICU stay. There was a non-significant trend toward a reduction in the requirement for mechanical ventilation and a significant improvement in biomarkers of systemic inflammation, renal function, and renal damage in blood and urine, suggesting that a systemic anti-inflammatory effect induced by the treatment resulted in fast recovery and prevention of further kidney damage.

No safety concerns were identified in either of these Phase 2 clinical studies with BiAP nor in the Phase 1 study in 51 healthy volunteers with recAP.

14.1.11 Change Number 11

The total blood volume collected from patients was specified.

14.1.11.1 Reason:

The Austrian ethics committee requested that the total blood volume taken was specified in the protocol.

14.1.11.2 Protocol Section:

Section 6.3.7 Laboratory Tests Performed by the Local Laboratory

Change to or Section Deleted:

Blood samples will be collected for local laboratory measurements at baseline, and at visits on Days 1, 3, 5, 7, 14, 21, and 28 (see Section 13.1 [Table 13-1] and Section 13.2

[Figure 13-1]). **Approximately 178 to 208 mL (35 to 42 teaspoons) of blood will be collected per patient in total during the study, including tests performed in the local and in the central laboratories.**

14.1.11.3 Protocol Section:

Section 6.3.8 Laboratory Test Performed by Central Laboratory

Change to or Section Deleted:

Urine samples for assessment of creatinine, urea, proteinuria, sodium, renal biomarkers, and purines will be shipped in one urine collection sample tube. If required for specific visits, further distribution to involved central laboratories will be managed centrally.

Approximately 178 to 208 mL (35 to 42 teaspoons) of blood will be collected per patient in total during the study, including tests performed in the local and in the central laboratories.

14.1.12 Change Number 12

Additional information regarding risks for women of childbearing potential has been added.

14.1.12.1 Reason:

To clarify the risks for women of childbearing potential.

14.1.12.2 Protocol Section:

Section 6.4 Pregnancy

Change to or Section Deleted:

The effect of recAP on pregnancies or infants is not known~~;~~**, as the safety of this drug during pregnancy has not been tested previously in either human or in animal studies. Because AP is present in the human placenta it is theoretically possible that anti-placental antibodies are developed after receiving study drug. Such antibodies could interfere with the ability to have a successful pregnancy. While development of anti-drug antibodies has not been seen to date in human studies, the safety experience**

with this drug is limited to only 37 subjects who received study drug and no data are available regarding effect of the drug on human reproduction.

Ttherefore, female patients who are pregnant or lactating/breastfeeding at time of screening or who intend to become pregnant within 28 days of enrolling into the study will be excluded from the study (see Section 4.1.2).

14.1.13 Change Number 13

References and justification for iohexol and para-aminohippuric acid were added.

14.1.13.1 Reason:

Following a discussion on 27 August 2014, the Arnhem-Nijmegen Region Committee of Human Subjects Research requested explanation of the added value of the iohexol and para-aminohippuric acid administration as well as references to literature.

14.1.13.2 Protocol Section:

Section 13.5.1 Introduction (Section 13.5 Appendix 5: Iohexol, Para-Aminohippuric Acid, and Exogenous Creatinine Clearance Protocol (Including Pharmacodynamics) for Selected Sites Only)

Change to or Section Deleted:

Accurate measurement of glomerular filtration **rate** (GFR) and effective renal plasma flow (ERPF) are valuable for evaluating kidney function and nephrotoxicity in a variety of clinical research and patient care settings. Accurate GFR determinations are recommended to appropriately assess the value of pharmacotherapeutic interventions aimed at improving tubular microvascular hemodynamics, inflammation and slowing the progression of renal disease.

The measure mostly used for the determination of the kidney function is the creatinine clearance as proxy for GFR. However, creatinine clearance is not fully representative in unstable situations, such as acute kidney injury, because it is influenced by various factors (e.g., diet, muscle mass, physical activity, and excretion and resorption in the kidney) and therefore results of creatinine clearance can be an over- or under-estimation. Creatinine clearance is routinely used to determine the function of

the kidneys irrespective of the drawbacks, since it is inexpensive, non-invasive, and easy to perform.

14.1.14 Change Number 14

Sample time points of effluent were corrected.

14.1.14.1 Reason:

Text was not in agreement with Schedule of Assessments.

14.1.14.2 Protocol Section:

Section 13.5.3 Iohexol and Para-Aminohippuric Acid Sampling

Change to or Section Deleted:

The sample time points of effluent will be at ~~t = 0 and~~ t = 360 minutes of the urine collection interval, analogous to the time points for serum creatinine determinations, as needed for endogenous creatinine clearance calculations. Two samples of 1 mL will be taken from the effluent and stored in Micronic 1.4-mL cryotubes at –80ºC, until batched shipments to the central laboratory are sent and further processing of one of each cryotube at the Hillcrest Medical Center can be done. The total volume of effluent will be recorded in the eCRF.

14.1.14.3 Protocol Section:

New Section 13.5.6 References (old Section 13.5.6 has been renumbered to 13.5.7, and old Section 13.5.7. has been numbered to 13.5.8)

Change to or Section Deleted:

Castagnet S, Blasco H, Vourc'h P, et al. Routine determination of GFR in renal transplant recipients by HPLC quantification of plasma iohexol concentrations and comparison with estimated GFR. J Clin Lab Anal. 2012;26(5):376-83.

Hirata-Dulas CA, Awni WM, Matzke GR, et al. Evaluation of two intravenous single-bolus methods for measuring effective renal plasma flow. Am J Kidney Dis. 1994;23(3):374-81.

Laroute V, Lefebvre HP, Costes G, et al. Measurement of glomerular filtration rate and effective renal plasma flow in the conscious beagle dog by single intravenous bolus of iohexol and p-aminohippuric acid. J Pharmacol Toxicol Methods. 1999;41(1)17-25.

Prescott LF, Freestone S, McAuslane JA. The concentration-dependent disposition of intravenous p-aminohippurate in subjects with normal and impaired renal function. Br J Clin Pharmacol. 1993;35(1):20-9.

14.1.15 Change Number 15

Address was added for PPD Global Central Laboratory in Belgium.

14.1.15.1 Reason:

Administrative change.

14.1.15.2 Protocol Section:

Table 10-1 Study Administrative Table

Change to or Section Deleted:

Central Laboratory~~y~~**ies**	PPD Global Central Laboratory 2 Tesseneer Drive Highland Heights, KY 41076 Phone: 859-781-8877 **PPD Global Central Laboratory** **Cluster Park** **Kleine Kloosterstraat 19** **B-1932 Zaventem, Belgium** **Phone: +32 2 725 2127**

14.1.16 Change Number 16

Addition of recent microbiological test results to the assessments done at screening in the Schedule of Assessments Table.

14.1.16.1 Reason:

To provide clarification on missing information from the Schedule of Assessments.

14.1.16.2 Protocol Section:

Section 13.1 Appendix 1: Schedule of Assessments; Table 13 1 Schedule of Assessments

Change to or Section Deleted:

Assessment	Screening (≤ 24 h)
…	
Recent microbial test results, if available	**X**

14.2 Amendment 2

The following changes from Protocol Version 2.0, including Amendment 1, dated 08 Oct 2014 were included in Protocol Version 3.0, including Amendment 2, dated 03 Feb 2016. Additions to the study protocol are shown in **bold** and deletions are shown in ~~strike through~~ text. Corrections of obvious typing errors or omissions and other editorial changes are not highlighted.

14.2.1 Change Number 1

Addition of new countries and sites and inclusion of text on analysis of patients during interim analysis period.

14.2.1.1 Reason:

Addition of new countries and sites projected for the study. Clarification on analysis of patients during interim analysis period.

14.2.1.2 Protocol Section:

Synopsis (Study Sites, Study design), Section 3.1 Study Design, Section 4.1 Selection of Study Population, Section 7.4 7.4 Sample Size Calculations

Change to or Section Deleted:

Approximately ~~50~~ **100** sites will participate in the **study**; **approximately 75 in the** European Union **and approximately 25 in** the United States **and Canada. Recruitment will continue during the interim analysis**.

A minimum of 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 ~~potentially~~ **continued** 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 **with an estimated 50 patients during the interim analysis**. Patients enrolled during Part 1 **and during the interim analysis** will be randomly assigned to receive, by 1-hour intravenous (IV) infusion, either placebo (Part 1; $n_1 = 30$) or one of 3 different doses of recAP (Part 1; $n_1 = 30$ 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 by 1-hour IV infusion once daily for 3 days (Days 1, 2, and 3). The interim analysis on the primary endpoint will be performed **on all data collected** after ~~all the patients~~ **the 120th** patient of Part 1 has completed the ~~first 7 days in Part 1~~ **Day 7 visit of the study** to select the dose to be administered in Part 2. The dose chosen will be the 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. In Part 2, patients will be randomly assigned to receive, by 1-hour IV infusion, either placebo ($n_2 = 85$) or the dose of recAP ($n_2 = 85$) selected during the interim analysis. **Patients recruited during the interim anaysis period to the dose selected in Part 2 will form part of the Part 2 populations, but those recruited to the doses that are not selected will be included in the Part 1 population.**

14.2.2 Change Number 2

Study design section has been amended with regard to inclusion and exclusion criteria.

14.2.2.1 Reason:

To fine tune the eligibility criteria in order to make the study more clear and workable for sites.

14.2.2.2 Protocol Section:

Section 4.1.1 Inclusion Criteria

Change to or Section Deleted:

2. Is aged 18 to ~~80~~ **85** years, inclusive
3. Is admitted to the ICU **or intermediate care unit**.

4. Has diagnosis of sepsis (<**96**~~72~~ hours prior to first study drug administration **or <72 hour prior to AKI diagnosis**), according to criteria defined by the American College of Chest Physicians/Society of Critical Care Medicine (Bone 1992]), based on:

    b. Have at least 2 of the following 4 SIRS criteria within ~~the~~ a timeframe of ~~48~~ **72** hours at time of ~~screening~~ **AKI diagnosis** ~~and 72 hours prior to first drug administration~~. Note: **it is not required that** symptoms are ~~not required to be~~ present simultaneously at study randomization:

5. Has first diagnosis of **AKI** ~~at screening~~, defined as ~~follows~~ any of the following:

    AKI Stage 1 or greater, according to the following Acute Kidney Injury Network (AKIN) criteria (Note: adjusted in regards to time-window):

    a. Increase **(absolute)** in serum creatinine > 26.2 µmol/L (0.30 mg/dL) **compared with a serum creatinine value** within the previous ~~24~~ **48** hours ~~prior to screening~~, or **presumed to have occurred in the previous 48 hours when compared to a reference* creatinine value**

    b. Increase **(relative)** in serum creatinine to > 150% (> 1.5–fold) **compared with a serum creatinine value in the previous 48 hours or presumed to have occurred in the previous 48 hours**, **when** ~~from~~ **compared to a reference* creatinine value** ~~within the previous 24 hours prior to screening~~ (in the absence of primary underlying renal disease). The reference creatinine value is **a** ~~the~~ serum creatinine value ~~according~~ **in** ~~to~~ the following order of preference:

~~6. AKI is likely attributable to the sepsis condition and not to other causes, e.g., contrast fluid, non-steroidal anti-inflammatory drugs (NSAIDs), or others.~~

6. When the diagnosis of AKI ~~was~~ **is** made according to one of the AKIN serum creatinine criteria (absolute or relative increase, see inclusion criteria 5a and 5b), continuing AKI needs to be confirmed by a confirmative serum creatinine measure (that is corrected for fluid administrations), **defined as no decrease in serum creatinine ≥ 26.2 µmol/L (≥0.30 mg/dL)**. The result must be available prior to randomization, within 24 hours after the primary AKI diagnosis so that administration of the first study treatment can be started within-24 hours after the first AKI diagnosis.

Section 4.1.2 Exclusion Criteria

Change to or Section Deleted:

2. Weighs more than ~~100~~ **115** kg (~~220~~ **253** lb).

6. Is already on dialysis (RRT) or ~~anticipated to receive~~ **a decision has been made to initiate** RRT within 24 hours **after planned start of** study drug **administration** ~~due to underlying disease~~.

21. ~~No confirmed diagnosis of continuing AKI within 24 hours after primary diagnosis (see inclusion criteria 7 and 8 for serum creatinine and urine output criteria, respectively)~~.

21. **Improvement in serum creatinine of at least 0.30 mg/dL or (26.2 µmol/L) prior to administration of the study drug.**

25. **Has active hematological malignancy**.

14.2.3 Change Number 3

Pharmacokinetic assessments were amended to reflect the subset of patients in whom they would be performed.

14.2.3.1 Reason:

Clarification was added that PK assessments would be performed for the first 120 patients in Part 1 only.

14.2.3.2 Protocol Section:

Synopsis (Exploratory Assessments)

Change to or Section Deleted:

PK analyses performed at all visits from Day 1 to Day 7 (inclusive) **in the first 120 patients from** Part 1 **only**

Section 2.2 Secondary Objectives

To investigate the pharmacokinetics (PK) of recAP in a subset ~~(Part 1)~~ of patients with SA-AKI (**in the first 120 patients from Part 1 only**).

Section 3.1.1 Rationale of Study Design

In **the first 120 patients from** Part 1 **only**, 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.

Section 6.3.8.3 Pharmacokinetic Assessments

Change to or Section Deleted:

The following PK samples will be taken from Day 1 to Day 7 (inclusive) **in the first 120 patients from Part 1 only** and exact sampling clock times will be recorded:

14.2.4 Change Number 4

Recording of concomitant medication use in the eCRF has been amended to introduce use of mean dosing by the system.

14.2.4.1 Reason:

Recording of concomitant medication use in the eCRF has been amended to introduce use of mean dosing by the system to avoid sites performing multiple manual calculations as much as possible. The list of IV medications has been updated.

14.2.4.2 Protocol Section:

Section 5.7 Concomitant Therapy, 6.3.12 Concomitant Medication

Change to or Section Deleted:

Concomitant medication use, including the drug name (brand or generic), dose, and dates of administration, will be recorded in the eCRF beginning on ~~Day 1~~ baseline to Day 28. Changes in dosage of any concomitant medication will be recorded in the eCRF, except for IV medications that undergo frequent adjustments during the same day or over time, such as **insulin**, vasopressors, **inotropics, diuretics, sedatives, metoprolol and potassium**. In these cases, only estimated mean daily dose **or mean daily dose as calculated by the system used**

will be recorded. Concomitant medications will include all prescription drugs, **including** vitamins and, minerals, herbal products, and over-the-counter medications. **Only brand names of nutritional products are to be recorded, while the single constituents of nutritional products need not be recorded.**

Administered resuscitation fluids should only be recorded as total daily volume in the concomitant medication section in the eCRF. Blood products should be recorded separately.

14.2.5 Change Number 5

Addition of height.

14.2.5.1 Reason:

Addition of height for calculation of the body mass index (BMI).

14.2.5.2 Protocol Section:

Section 6.3.1 Demographics and Medical History

Change to or Section Deleted:

Demographics and medical history should be recorded at baseline (see Section 13.1 [Table 13-1] and Section 13.2 [Figure 13-1]). **Height will be collected at baseline for calculation of body mass index (BMI; body weight (kg) / [height (m)]^2).**

14.2.6 Change Number 6

Clarification in weight.

14.2.6.1 Reason:

Clarification in weight for calculation of study drug dose and treatment administered.

14.2.6.2 Protocol Section:

Synopsis (Study Drug, Dosage, and Route of Administration), Section 5.2

Change to or Section Deleted:

Patients weighing between 95 to 115 kg will receive the same dose as that for patients weighing 100 kg.

Section 6.3.5 Weight

Change to or Section Deleted:

For determination of study drug dosage, body weight (in kg or lb) will be ~~measured or~~ estimated ~~at screening~~. **The hospital admission weight (in kg or lb) will be used for study drug preparation and** ~~and dosing during the 3 days of study drug administration~~ **for calculation of fluid-corrected serum creatinine**.

14.2.7 Change Number 7

Addition of a new analysis set (ITT Part 1 interim set). Addition of other analysis sets to reflect the current SAP.

14.2.7.1 Reason:

Added a new analysis set to adequately distinguish between the Part 1 patients and the subset of these that are included in the interim analysis.

14.2.7.2 Protocol Section:

Section 7 Analysis Set

Change to or Section Deleted:

- **ITT Part 1 interim set: 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.**

- Per-protocol (PP) Day 1-7 **combined** set: all patients who were randomly assigned to a study drug, had no significant protocol deviations (e.g., wrong drug volume received, administration of prohibited concomitant medication, patient did not meet all inclusion criteria or met 1 or more exclusion criteria) and had complete data 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.

- **Per-protocol (PP) Day 1-7 Part 2 set: all patients who were randomly assigned to a study drug after the conclusion of Part 1 of the study, had no significant protocol deviations (e.g., wrong drug volume received, administration of prohibited concomitant medication, patient did not meet all inclusion criteria or met 1 or more exclusion criteria) and had complete data 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.

14.2.8 Change Number 8

Change in cut-off date for capture of unrelated AEs and SAEs.

14.2.8.1 Reason:

Change in cut-off date for capture of unrelated AEs and SAEs to the end of study day.

14.2.8.2 Protocol Section:

Section 6.3.14.2 Eliciting and Documenting Adverse Events

Change to or Section Deleted:

Adverse events will be collected and assessed from the time the patient signs the ICF ~~until 30 days after the last dose of study drug or later~~ **Day 28 visit** for **all AEs (including SAEs)** ~~possibly, probably, or definitely related to study drug~~ **regardless of its relationship to study drug**. Any AE that occurs during or after the first dose of study drug is considered treatment emergent.

14.2.9 Change Number 9

Update to data to be reviewed by DMC

14.2.9.1 Reason:

Clarification in data to be reviewed by DMC

14.2.9.2 Protocol Section:

Section 10.1.1 External Data Monitoring Committee

Change to or Section Deleted:

An independent DMC will review the results of the interim analysis and select the optimum recAP dose for Part 2 of the study (see Section 7.7.5). **The DMC will conduct three additional reviews of the safety data by teleconference once the first 7 days of laboratory data are available for the following: 75 patients in Part 1, 60 patients in Part 2, and 125 patients in Part 2. In each case, the milestone will be patients with at least 7 days of laboratory data. Additional electronic reviews will be conducted of unblinded AE listings after 25, 50 and 100 patients in Part 1 and 30 and 90 patients in Part 2. The DMC can also request ad hoc reviews**. Accumulating safety and efficacy data will be reviewed at predefined milestones during Part 1 and Part 2 of the study by the independent DMC as described in the DMC charter as follows:

- **Electronic Review 25 subjects**
- **Electronic Review 50 subjects**
- **Teleconference 75 subjects**
- **Electronic Review 100 subjects**
- **Face to Face Meeting-interim analysis-120 subjects**
- **Ad hoc Review- (if needed) between 90-120 additional subjects**
- **Teleconference 60 additional subjects from Part 2 (at least 180 total)**
- **Electronic Review 90 additional subjects from Part 2 (at least 210 total)**
- **Teleconference 125 additional subjects from Part 2 (at least 245 total)**
- **Ad hoc Review- (if needed) between 125-170 subjects from Part 2 (at least 245-290 total)**.

14.2.10 Change Number 10

Address was updated for PPD Medical Monitor in European Union.

14.2.10.1 Reason:

Administrative change.

14.2.10.2 Protocol Section:

Cover page, Table 10-1 Study Administrative Table

Change to or Section Deleted:

European Union:
Hanna Orr, MD
Associate Medical Director, Pharmacovigilance,
PPD Office Warsaw (Home Based)
49 Domaniewska Street, Trinity Park III; 1st floor
02-672 Warsaw, Poland
Phone: +48 781811328

European Union:
Hanna Orr, MD
Associate Medical Director, Pharmacovigilance,
PPD
~~Fleming House, Strathclyde Business Park~~
~~Bellshill, Lanarkshire, ML4 3NJ, UK~~
~~Phone: +44 (1223) 374240~~

14.2.11 Change Number 11

References to para-aminohippuric acid (PAH) were deleted.

14.2.11.1 Reason

References to para-aminohippuric acid (PAH) were deleted as it is commercially not available anymore.

14.2.11.2 Protocol Section:

Section 6.3.8.7 Glomerular Filtration Rate

Change to or Section Deleted:

A limited number of sites **only** will simultaneously determine GFR with iohexol administration and blood sampling (considered the golden standard) to globally compare and assess correlation of GFR and calculated endogenous creatinine clearance. ~~Para-~~

~~aminohippuric acid (PAH) will be administered and samples taken to assess renal blood flow.~~ In addition, at these sites samples from the effluent from patients on RRT will be taken to assess exogenous (machine) creatinine clearance. Detailed instructions to these sites for iohexol and ~~PAH assessments and~~ exogenous creatinine clearance are provided in Section 13.5.

Section 13.5 Appendix 5: Iohexol and Exogenous Creatinine Clearance Protocol (Including Pharmacodynamics) for Selected Sites Only (Section 13.5.1, Section 13.5.2, Section 13.5.3, Table 13-3 [Section 13.5.7], Section 13.5.8)

Change to or Section Deleted:

Accurate measurement of glomerular filtration rate (GFR) ~~and effective renal plasma flow (ERPF) are~~ is valuable for evaluating kidney function and nephrotoxicity in a variety of clinical research and patient care settings.

Marker compounds such as iothalamate, iohexol, or inulin have been used extensively to measure GFR~~, while ERPF is most commonly measured using para-aminohippuric acid (PAH)~~.

~~Para-aminohippuric acid is an ideal marker for estimating ERPF since it is freely filtered at the glomerulus and undergoes extensive secretion and negligible reabsorption within renal tubules. However, approximately 20% of PAH is metabolized to acetyl-para-aminohippuric acid, which is also extensively secreted.~~

Iohexol ~~and Para-Aminohippuric Acid~~ Dosing

~~PAH: A single intravenous bolus injection of 400 mg PAH will be given over 5 minutes on Day 1 and on Day 7 or discharge from the intensive care unit (ICU), whichever comes first. The bolus will be administered at the start of the 6 ± 1 hour urine collection interval.~~

Iohexol ~~and Para-Aminohippuric Acid~~ Sampling

For the ~~simultaneous~~ measurement of ~~both~~ iohexol ~~and PAH~~, 2-mL blood samples will be collected in EDTA anticoagulated Vacutainer® tubes at the following time points: prior to ~~PAH and~~ iohexol bolus administration and at 60, 120, and 360 minutes.