Document Type: Final Protocol

Document Date: 25 October 2021

Study Title: A Randomized, Double-Blind, Placebo-Controlled, Two-Arm Parallel Group, Multi-Center Phase 3 Pivotal Trial to Investigate the Efficacy and Safety of Recombinant Human Alkaline Phosphatase for Treatment of Patients with Sepsis-Associated Acute Kidney Injury

Protocol Reference Number: AP-recAP-AKI-03-01

NCT Number: NCT04411472

Title Page

Protocol Title: A Randomized, Double-Blind, Placebo-Controlled, Two-Arm Parallel-Group, Multi-Center Phase 3 Pivotal Trial to Investigate the Efficacy and Safety of Recombinant Human Alkaline Phosphatase for Treatment of Patients with Sepsis-Associated Acute Kidney Injury

Short Title: Recombinant human alkaline phosphatase SA-AKI survival trial
Trial acronym: REVIVAL

Protocol Number: AP-recAP-AKI-03-01
Amendment Number: Not applicable

Compound Name: Recombinant human Alkaline Phosphatase
International Nonproprietary Name: Ilofotase alfa

Sponsor Name and Legal Registered Address:
AM-Pharma B.V.
Stadsplateau 6
3521 AZ Utrecht
The Netherlands

Regulatory Agency Identifying Numbers: US IND Number 117605
EudraCT Number 2019-004625-24

Approval Date: October 25, 2021
Date and Version of Previous Protocol: May 19, 2020

Sponsor Signatory:

PPD

AM PHARMA

Confidential

The information in this document is confidential and proprietary to AM-Pharma. No disclosure, dissemination or distribution of this document is permitted without the prior written consent of AM-Pharma.

Table of Contents

Title Page 1

Table of Contents 3

List of Abbreviations and Protocol-specific Definitions 8

1 Protocol Summary 11

1.1 Protocol Synopsis 11

1.2 Trial Design and Trial Flowchart for the Individual Patient 23

1.3 Schedule of Activities 27

2 Introduction 31

2.1 Trial Rationale 31

2.2 Background 31

2.2.1 Sepsis and Acute Kidney Injury 31

2.2.2 Alkaline Phosphatase 32

2.2.3 Recombinant Human Alkaline Phosphatase (recAP) 33

2.2.4 Pre-clinical and Clinical Studies with Recombinant Human Alkaline Phosphatase (recAP) 33

2.3 Benefit/Risk Assessment 34

3 Objectives and Endpoints 36

4 Trial Design 38

4.1 Overall Design 38

4.2 Scientific Rationale for Trial Design 40

4.3 Justification for Dose 41

4.4 End of Trial Definition 41

5 Trial Population 41

5.1 Inclusion Criteria 42

5.2 Exclusion Criteria 43

5.3 Lifestyle Considerations 47

6 Trial Drug 47

6.1 Trial Drugs Administered 47

6.2 Preparation/Handling/Storage/Accountability 48

6.2.1 Trial Drug Preparation 48

6.2.2 Trial Drug Administration 49
6.2.3 Trial Drug Handling and Storage 49
6.2.4 Trial Drug Accountability 49
6.3 Measures to Minimize Bias: Randomization and Blinding 50
6.3.1 Randomization 50
6.3.2 Blinding 50
6.3.2.1 Breaking the Blind 51
6.4 Trial Drug Compliance 51
6.5 Prior and Concomitant Medications 52
6.5.1 Concomitant Medications to be Avoided 52
6.6 Dose Modification 52
6.7 Intervention After the End of the Trial 52
7 Discontinuation of Trial Drug and Patient Discontinuation 53
7.1 Discontinuation of Trial Drug 53
7.2 Patient Withdrawal from the Trial 53
7.3 Loss of Patients to Follow-Up 54
8 Screening and Enrollment, Trial Days, Trial Assessments and Procedures 54
8.1 Pre-screening, Screening and Enrolment 54
8.2 Trial Days 55
8.2.1 Pre-screening, Screening and Baseline 55
8.2.2 Day 1 to Day 3: Treatment Days 55
8.2.3 Days 4, 5, 6, 7, 28, 90, and 180 Assessments and Procedures 56
8.3 Trial Assessments and Procedures 56
8.3.1 Eligibility/Screening Assessments 56
8.3.1.1 Diagnosis of Sepsis Requiring Vasopressor Therapy 56
8.3.1.2 Diagnosis of Acute Kidney Injury 57
8.3.1.3 Informed Consent 57
8.3.1.4 Pregnancy Test 57
8.3.2 Demographics and Other Baseline Assessments 57
8.3.2.1 Demographics 57
8.3.2.2 Medical History 58
8.3.2.3 Weight and Height 58
8.3.2.4 12-lead Electrocardiogram 58
8.3.2.5 Acute Physiology and Chronic Health Evaluation (APACHE) II Score 58
8.3.2.6 SOFA Score 59

8.3.2.7 KDIGO AKI Stage 59
8.3.2.8 Site of Infection and Pathogen Causing Sepsis 59
8.3.2.9 Other Baseline Assessments 59
8.3.3 Efficacy Assessments 59
8.3.3.1 Mortality 59
8.3.3.2 Serum or Plasma Creatinine Levels and eGFR Calculation 59
8.3.3.3 KDIGO AKI Stage 60
8.3.3.4 Modified Sequential Organ Failure Assessment Score (mSOFA score) 61
8.3.3.5 Mechanical Ventilation 61
8.3.3.6 Renal Replacement Therapy 62
8.3.3.7 Use of Vasopressors and/or Inotropic Drugs 62
8.3.3.8 Quality of Life EQ-5D-5L Questionnaire 62
8.3.3.9 Functional Status and Residence 63
8.3.3.10 Length of Intensive Care Unit and Hospital Stay and Rehospitalization 63
8.3.3.11 Biomarkers 63
8.3.4 Safety Assessments 64
8.3.4.1 Prior and Concomitant Medication 64
8.3.4.2 Clinical Chemistry and Hematology 64
8.3.4.3 Anti-Drug Antibodies (ADA) 65
8.3.4.4 Adverse Events 66
8.3.4.4.1 Time Period for Collecting AE and SAE Information 66
8.3.4.4.2 Follow-up of AEs and SAEs 67
8.3.4.4.3 Regulatory Reporting Requirements for SAE 67
8.3.4.4.4 Pregnancy 67
8.3.4.4.5 Laboratory Analyses 68
8.3.5 Others 68
8.3.5.1 Pharmacokinetics 68
8.4 Medication Errors and Treatment of Overdose 68
8.5 Genetics 68
9 Statistical Considerations 68
9.1 Analysis Sets 69
9.2 Statistical Analyses 69
9.2.1 Efficacy Analyses 69
9.2.1.1 Analysis of the Primary Efficacy Endpoint 70
9.2.1.1.1 Statistical Analysis 70
9.2.1.1.2 Subgroup Analyses 71

9.2.1.2 Analysis of Secondary Efficacy Endpoints 71
9.2.1.2.1 Major Adverse Kidney Events (MAKE) 90 71
9.2.1.2.2 Days Alive and Free of Organ Support Through Day 28 71
9.2.1.2.3 Days Alive and Out of the ICU Through Day 28 72
9.2.1.2.4 Time to Death Through Day 90 72
9.2.1.3 Tertiary/Exploratory Endpoints 73
9.2.1.4 Safety Endpoints 73
9.2.2 Other Analyses 74
9.2.3 Population Pharmacokinetics 74
9.3 Interim Analyses 74
9.4 Sample Size Determination 75
10 Supporting Documentation and Operational Considerations 77
10.1 Appendix 1: Regulatory, Ethical, and Trial Oversight Considerations 77
10.1.1 Regulatory and Ethical Considerations 77
10.1.2 Financial Disclosure 78
10.1.3 Informed Consent Process 78
10.1.4 Data Protection 79
10.1.5 Committees Structure 79
10.1.6 Dissemination of Clinical Trial Data 80
10.1.7 Data Collection, Quality Assurance, and Management 80
10.1.8 Source Documents 82
10.1.8.1 Investigator Documentation 83
10.1.9 Trial and Site Closure 83
10.1.10 Records Retention 83
10.1.11 Publication Policy 84
10.1.12 Trial Administration 84
10.1.13 Protocol Approval and Amendment and Protocol Deviations 86
10.1.13.1 Protocol and Protocol Amendments 86
10.1.13.2 Protocol Deviations 86
10.1.13.3 Access to Source Data 86
10.2 Appendix 2: Adverse Events: Definitions and Procedures for Recording, Evaluating, Follow-up, and Reporting 88
10.3 Appendix 3: Statistical Design Report 95
11 References 103
Investigator Agreement Page 106

List of Tables

Table 1: Gender and Race Corrected Cut-off Values for Serum or Plasma CR Based on 1.5 Times Estimated Normal Values for Age Group 16

Table 2: Lan DeMets O'Brien-Fleming Nominal One-Sided *p*-values Required for Early Success at each Interim Analysis 22

Table 3: Schedule of Activities 27

Table 4: Gender and Race Corrected Cut-off Values for Serum or Plasma CR Based on 1.5 Times Estimated Normal Values for Age Group[2] 43

Table 5: Trial Drugs Administered 48

Table 6: KDIGO AKI Staging 60

Table 7: Protocol-Required Safety Laboratory Assessments 65

Table 8: Nominal 1-Sided Significance Levels for Success at Interim and Final Analyses (per the Lan-DeMets Approximation of the O'Brien-Fleming Alpha Spending Function) . 75

Table 9: Operating Characteristics with Maximum Sample Size of 1,400 and Chosen Group-sequential Design 76

Table 10: Personnel and Organizations Responsible for Trial Conduct 85

Table of Figures

Figure 1: Trial design 23

Figure 2: Timelines for Eligibility 25

Figure 3: Trial Flow for the Individual Patient 26

List of Abbreviations and Protocol-specific Definitions

List of abbreviations

ADA	anti-drug antibodies
ADP	adenosine diphosphate
AE	adverse event
AIDS	Acquired Immune Deficiency Syndrome
AKI	acute kidney injury
AMP	adenosine monophosphate
AP	alkaline phosphatase
APACHE	Acute Physiology and Chronic Health Evaluation
ATP	adenosine triphosphate
cAMP	cyclic adenosine monophosphate
CFR	Code of Federal Regulations
CI	confidence interval
CKD	chronic kidney disease
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration
COVID-19	Corona Virus Disease 2019
CPR	cardiopulmonary resuscitation
CR	Creatinine
CRO	Contract research organization
CTR	Clinical Trial Report
DAMPS	damage-associated molecular pattern molecules
DBL	database lock
DMC	Data Monitoring Committee
ECC	endogenous creatinine clearance
ECG	electrocardiogram
EDC	electronic data capture
EQ-5D-5L	EuroQoL-5-Dimensions–5 Levels
eCRF	electronic case report form
eGFR	estimated glomerular filtration rate
EOT	end of trial
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GCS	Glasgow Coma Scale

h	hour
IB	Investigator Brochure
ICF	informed consent form
ICH	International Conference on Harmonisation
ICU	intensive care unit
IEC	Independent Ethics Committee
IND	Investigational New Drug
IRB	Institutional Review Board
IRT	interactive response technology
IV	intravenous
KDIGO	Kidney Disease Improving Global Outcomes
KM	Kaplan-Meier
LOS	length of stay
LPS	lipopolysaccharide
LTFU	lost to follow-up
MAD	Multiple ascending dose
MAKE	Major Adverse Kidney Events
MedDRA	Medical Dictionary for Regulatory Activities
mITT	modified Intent-to-Treat
mSOFA	Modified Sequential Organ Failure Assessment score (excluding Glasgow Coma Score)
MV	mechanical ventilation
N	number
PAMPS	pathogen-associated molecular pattern molecules
PD	pharmacodynamic(s)
PK	pharmacokinetic(s)
PK-PD	pharmacokinetic-pharmacodynamic
PP_{max}	maximum sample size
QALY	quality-adjusted life years
QoL	Quality of Life
recAP	recombinant human alkaline phosphatase
RRT	renal replacement therapy
SA-AKI	sepsis-associated acute kidney injury
SAD	Single ascending dose
SAE	serious adverse event

SAP	Statistical Analysis Plan
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
SIRS	Systemic inflammatory response syndrome
SoA	schedule of activities
SOFA	Sequential Organ Failure Assessment
SOP	standard operating procedures
SUSAR	suspected unexpected serious adverse reaction
TEAE	treatment emergent adverse event
TLR4	toll-like receptor 4
US	United States
VAS	visual analogue scale
WOCBP	women of childbearing potential

Protocol-specific Definitions

Evaluable patient	Treated patient in the main trial population who has reached Day 28
'Moderate' CKD	A pre-AKI reference eGFR ≥25 and <45 mL/min/1.73 m^2
Norepinephrine equivalent	1 µg norepinephrine is equivalent to 1 µg epinephrine or 100 µg dopamine[1]
Pre-AKI reference value	The pre-AKI reference value refers to the patient's usual CR/eGFR level before developing AKI. The pre-AKI reference value is defined as the median of the 3 most recent CR/eGFR values in the past 12 months before developing AKI. For patients with known CKD, the median of values covering at least 3 months should be used. If less than 3 values are available, the most recent value is to be used.
'Severe' CKD	A pre-AKI reference eGFR <25 mL/min/1.73 m^2
Vasopressors	The following drugs are considered vasopressors: norepinephrine, epinephrine, dopamine, phenylephrine, vasopressin, and angiotensin II. Following the initial one hour on at least 0.1 µg/kg/min norepinephrine or equivalent, any IV dose of vasopressor counts as vasopressor therapy.

1 Protocol Summary

1.1 Protocol Synopsis

Protocol Title:

A Randomized, Double-Blind, Placebo-Controlled, Two-Arm Parallel-Group, Multi-Center Phase 3 Pivotal Trial to Investigate the Efficacy and Safety of Recombinant Human Alkaline Phosphatase for Treatment of Patients with Sepsis-Associated Acute Kidney Injury

Coordinating Investigator:

Peter Pickkers, MD, PhD, Dept Intensive Care Medicine, Radboud University Medical Center, Nijmegen, Netherlands

Sponsor:	**Sponsor Trial No.:**
AM-Pharma B.V.	AP-recAP-AKI-03-01
Planned Trial Period:	**Clinical Phase:**
First patient first visit: Q4 2020 Last patient last visit: Q1 2024	3 (Pivotal)

Rationale:

Sepsis is the leading cause of acute kidney injury (AKI) and a major cause of death. Patients with sepsis associated AKI (SA-AKI) have a high mortality and morbidity and are at risk of developing chronic kidney disease (CKD). Alkaline phosphatase (AP) is a homodimeric endogenous enzyme present in many cells and organs, e.g., intestines, placenta, liver, bone, kidney, and granulocytes. It exerts detoxifying effects through dephosphorylation of endotoxins; pathogen-associated molecular pattern molecules (PAMPS e.g., lipopolysaccharide [LPS]) and damage-associated molecular pattern molecules (DAMPS e.g., adenosine tri- and di-phosphate [ATP and ADP]). In animal models of sepsis and AKI, administration of AP attenuates the inflammatory response, improves renal function, and/or reduces mortality.

AM-Pharma B.V. (hereafter referred to as the Sponsor) is developing AP as a novel, recombinant chimeric human AP medicinal product, called recombinant human alkaline phosphatase (recAP) to be used as an intravenous (IV) infusion for the treatment of SA-AKI. In the Phase 2 trial STOP-AKI, a survival benefit was observed in the two highest dose groups, 0.8 mg/kg and 1.6 mg/kg groups, compared to the placebo group. No safety or tolerability concerns were observed for any of the doses tested (0.4, 0.8 and 1.6 mg/kg). The 1.6 mg/kg recAP dose was selected for this Phase 3 trial based on the significant survival benefit observed. Pharmacokinetic/pharmacodynamic (PK-PD) simulations also confirmed this dose to have the most pronounced treatment effect.

The primary objective of this Phase 3 trial is to confirm the mortality benefit seen in the Phase 2 trial STOP-AKI by demonstrating a reduction in 28-day all-cause mortality in patients with SA-AKI treated with 1.6 mg/kg recAP.

Objectives and Endpoints:

OBJECTIVES	ENDPOINTS
Primary	
To demonstrate an effect of recombinant human alkaline phosphatase (recAP) on 28-day all-cause mortality.	28-day all-cause mortality.
Secondary	
To investigate the effect of recAP on long-term Major Adverse Kidney Events (MAKE).	MAKE 90: dead by Day 90 *or* on Renal Replacement Therapy (RRT) at Day 90 *or* ≥25% decline in estimated glomerular filtration rate (eGFR) on both Day 28 and Day 90 relative to the known or assumed pre-AKI reference level.
To investigate the effect of recAP on use of organ support, i.e., mechanical ventilation (MV), RRT, vasopressors or inotropes.	Days alive and free of organ support through Day 28, i.e., days alive with no MV, RRT, vasopressors or inotropes (with death within 28 days counting as zero days).
To investigate the effect of recAP on length of stay (LOS) in intensive care unit (ICU).	Days alive and out of the ICU through Day 28 (with death within 28 days counting as zero days).
To investigate the effect of recAP on 90-day all-cause mortality.	Time to death through Day 90.
Tertiary/Exploratory	
To investigate the effect of recAP on 180-day all-cause mortality.	Time to death through Day 180.
To investigate the effect of recAP on organ function in the first week.	Change in total and individual organ failure scores through Day 7 (based on the modified Sequential Organ Failure Assessment [mSOFA] scores, defined as the SOFA score without the Glasgow Coma Scale [GCS] component).

OBJECTIVES	ENDPOINTS
To investigate the effect of recAP on short- and long-term renal function.	Days alive and free of RRT through Day 28 (with death within 28 days counting as zero days). MAKE 28: dead by Day 28 *or* on RRT at Day 28 *or* ≥25% decline in eGFR on both Day 7/ICU discharge (whichever comes first) and Day 28 relative to the known or assumed pre-AKI reference level. Patients alive and free of AKI on Day 7/ICU discharge (whichever comes first) and on Day 28. Patients alive and free of new onset CKD or worsening of CKD (defined as any increase in CKD Stage) on Day 90.
To investigate the effects of recAP on cardiovascular dysfunction.	Days alive and free of vasopressor and inotropes through Day 28 (with death within 28 days counting as zero days).
To investigate the effect of recAP on pulmonary function	Days alive and free of MV through Day 28 (with death within 28 days counting as zero days).
To investigate the effect of recAP on LOS in hospital and rehospitalization.	Days alive and out of the hospital through Day 90 (with death within 90 days counting as zero days). Incidence of at least one rehospitalization at any hospital through Day 90.
To investigate the effect of recAP on Quality of Life (QoL).	Change in index values, quality-adjusted life years (QALY) and visual analogue scale (VAS) score based on the EuroQoL-5-Dimensions-5Levels (EQ-5D-5L) questionnaire at Day 28, Day 90 and Day 180.
To investigate the effects of recAP on urinary excretion of purines.	The urinary levels of purines (ATP, ADP, adenosine monophosphate [AMP], cyclic adenosine monophosphate [cAMP], and adenosine) through Day 4 at selected sites.
Pharmacokinetics	

OBJECTIVES	ENDPOINTS
To investigate the pharmacokinetic (PK) properties of recAP.	Population PK

Safety	
To investigate the safety and tolerability of recAP.	Generation of anti-recAP antibodies on Day 28 and Day 90. Incidence of adverse events (AEs) and serious AEs (SAEs) through Day 28.

Overall Design:

This is a Phase 3, multi-center, randomized, double-blind, placebo-controlled, 2-arm parallel-group-sequential design pivotal trial in which patients with SA-AKI will be randomly assigned in a 1:1 ratio to either placebo or to 1.6 mg/kg recAP. Randomization will be stratified by:

- 'Moderate' CKD defined as a pre-AKI reference eGFR ≥25 and <45 mL/min/1.73 m^2
  - Yes
  - No
- Baseline mSOFA score, i.e., excluding the GCS part.
  - mSOFA score ≤9
  - mSOFA score >9
- Clinical site

An additional cohort of patients with SA-AKI and proven or suspected SARS-CoV-2 will be included in the trial. This cohort will also be randomly assigned in a 1:1 ratio to either placebo or to 1.6 mg/kg recAP. Randomization for this cohort will be stratified by:

- Baseline mSOFA score, i.e., excluding the GCS part.
  - mSOFA score ≤9
  - mSOFA score >9
- Clinical site

Based on this, we define three distinct SA-AKI trial populations:

1. **The main trial population:** Patients with a pre-AKI reference eGFR ≥45 mL/min/1.73 m^2 and no proven or suspected SARS-CoV-2 at time of randomization.
2. **A 'moderate' CKD population:** Patients with a pre-AKI reference eGFR ≥25 and <45 mL/min/1.73 m^2 and no proven or suspected SARS-CoV-2 at time of randomization.

3. **A Corona Virus Disease 2019 (COVID-19) population:** Patients with proven or suspected SARS-CoV-2 at time of randomization with or without 'moderate' CKD. For patients in this population, COVID-19 should be the main cause of SA-AKI.

Analysis of data from each population will be performed and presented separately. Formal analyses (incl. interim analyses), hypothesis testing and descriptive analyses will be performed on data from the main trial population, whereas only descriptive statistics (including an estimate of the treatment effect, two-sided 95% confidence intervals and one-sided p-value) will be presented for the 'moderate' CKD population and the COVID-19 population. Additional exploratory analyses for each of the three populations as well as for the pooled main trial population plus 'moderate' CKD population, and the pooled main trial population plus COVID-19 population are planned and will be described in the Statistical Analysis Plan (SAP).

There will be a maximum of four interim analyses during the trial and enrolment continues during the interim analysis. The interim analyses will take place after approximately 400, 700, 850, and 1,000 evaluable patients (i.e., treated patients in the main trial population who have reached Day 28). At the first interim analysis, the trial may be stopped for futility. At subsequent interim analyses, the trial may be stopped for futility or for success (i.e., early demonstration of superiority of recAP over placebo on 28-day all-cause mortality).

An independent Data Monitoring Committee (DMC) will evaluate safety data at regular intervals throughout the trial and notify the Sponsor and the Trial Steering Committee in case of safety concerns that lead to a recommendation to stop or modify the trial. Data from patients in the 'moderate' CKD population and in the COVID-19 population will also be included at each safety review.

The DMC will review the interim analysis reports and notify the Sponsor and the Trial Steering Committee in case a futility or success threshold is reached. The DMC will follow an agreed charter and will provide written communication to Sponsor and the Trial Steering Committee on its recommendation on trial continuation or discontinuation based on safety data and the interim analysis reports. More details of the safety data reviews and the interim analyses will be provided in the DMC charter and DMC SAP, which will both be finalized before the first safety data review.

Target Population:

The target patient population consists of adult patients in the ICU or intermediate care unit with sepsis and new, recent onset AKI. Consecutive adult patients with sepsis requiring vasopressor therapy will be systematically screened for AKI as soon as possible following the start of vasopressor treatment. In order to enroll a typical, random sample, reflecting the entry criteria, informed consent will be sought in all patients with AKI and no exclusion criteria.

Inclusion Criteria

To be eligible for this trial, a patient must meet all of the following inclusion criteria:

1. 18 years or older.
2. In the ICU or intermediate care unit for clinical reasons.
3. Have sepsis requiring vasopressor (norepinephrine, epinephrine, dopamine, phenylephrine, vasopressin, or angiotensin II) therapy, i.e.:

a) suspected or proven bacterial or viral infection

and

b) on vasopressor therapy (≥0.1 µg/kg/min norepinephrine or equivalent) for sepsis-induced hypotension for at least one hour despite adequate fluid resuscitation according to clinical judgement. Following the initial one hour on at least 0.1 µg/kg/min norepinephrine or equivalent, any dose of vasopressor counts as vasopressor therapy.

4. Have AKI according to at least one of the below Kidney Disease Improving Global Outcomes (KDIGO) criteria, a) to d):
   a) An absolute increase in serum or plasma creatinine (CR) by ≥0.3 mg/dL (≥26.5 µmol/L) within 48 hours

      or

   b) A relative increase in CR to ≥1.5 times pre-AKI reference CR value (see Section 8.3.3.3), which is known or presumed to have occurred within prior 7 days

      or

   c) A decrease in urinary output to <0.5 mL/kg/hour for a minimum of 6 hours following adequate fluid resuscitation

      or

   d) If the patient does not have a known history of CKD and there is no pre-AKI reference CR value (see Section 8.3.3.3) available from the past 12 months: a CR value greater or equal to the levels presented in Table 1, with the increase in CR presumed to have occurred within prior 7 days.

Table 1: Gender and Race Corrected Cut-off Values for Serum or Plasma CR Based on 1.5 Times Estimated Normal Values for Age Group[2]

Age (years)	Black males mg/dL (µmol/L)	Other males mg/dL (µmol/L)	Black females mg/dL (µmol/L)	Other females mg/dL (µmol/L)
20-24	2.3 (200)	2.0 (173)	1.8 (159)	1.5 (132)
25-29	2.3 (200)	1.8 (159)	1.7 (146)	1.5 (132)
30-39	2.1 (186)	1.8 (159)	1.7 (146)	1.4 (120)
40-54	2.0 (173)	1.7 (146)	1.5 (132)	1.4 (120)
55-65	2.0 (173)	1.7 (146)	1.5 (132)	1.2 (107)
>65	1.8 (159)	1.5 (132)	1.4 (120)	1.2 (107)

5. Provision of signed and dated informed consent form (ICF) in accordance with local regulations.

Exclusion Criteria

A patient who meets any of the following criteria is excluded from participation in this trial:

1.

a) At sites where enrolment of 'moderate' CKD patients is allowed, patients with 'severe' CKD defined as a pre-AKI reference eGFR <25 mL/min/1.73 m^2 are excluded.
   - For patients with known CKD, the most recent eGFR prior to index hospitalization needs to be documented as ≥25 mL/min/1.73 m^2.
   - For patients with known CKD but no known eGFR prior to hospitalization, presentation eGFR between 25-60 mL/min/1.73 m^2 can also be used to rule out 'severe' CKD.

b) At sites where enrolment of 'moderate' CKD patients is NOT allowed, patients with 'moderate' and 'severe' CKD defined as a pre-AKI reference eGFR <45 mL/min/1.73 m^2 are excluded.
   - For patients with known CKD, the most recent eGFR prior to index hospitalization needs to be documented as ≥45 mL/min/1.73 m^2.
   - For patients with known CKD but no known eGFR prior to hospitalization, presentation eGFR between 45-60 mL/min/1.73 m^2 can also be used to rule out 'moderate' and 'severe' CKD.

2. Advanced chronic liver disease, defined as a Child-Pugh score of 10 to 15 (Class C).
3. Acute pancreatitis without proven infection.
4. Urosepsis related to suspected or proven urinary tract obstruction.
5. Main cause of AKI not sepsis.
6. Proven or suspected SARS-CoV-2 infection. *This exclusion criterion does not apply to patients in the COVID-19 population, in which COVID-19 should be the main cause of SA-AKI.*
7. Severe burns requiring ICU treatment.
8. Severely immunosuppressed, e.g. due to:
   - hematopoietic cell transplantation within past 6 months prior to Screening or acute or chronic graft-versus-host disease
   - solid organ transplantation
   - leukopenia not related to sepsis, i.e., preceding sepsis
   - Human Immunodeficiency Virus (HIV)/Acquired Immune Deficiency Syndrome (AIDS)
   - receiving chemotherapy within 30 days prior to Screening.
9. At high risk of being lost to follow-up (LTFU), e.g., due to known current or recent (within the last 6 months) IV drug abuse or known to be homeless.
10. Limitations to use of MV, RRT or vasopressors and inotropes (NOTE: limitation of cardiopulmonary resuscitation (CPR) only is not an exclusion criterion).
11. Previous administration of recAP.

12. Use of a non-marketed drug within the last month or concurrent or planned participation in a clinical trial for a non-marketed drug or device. (NOTE: Co-enrollment or concurrent participation in observational, non-interventional trials using no protocolized treatments or procedures are always allowed. Co-enrollment or concurrent participation in trials using protocolized treatments or procedures, e.g. blood draws, requires pre-approval by the TSC).
13. Current or planned extracorporeal membrane oxygenation (ECMO).
14. On RRT >24 hours before start of trial drug.
15. No longer on vasopressor therapy at time of randomization.
16. On continuous vasopressor therapy for >72 hours before start of trial drug.
17. Estimated glomerular filtration rate (eGFR) >60 mL/min/1.73 m^2 based on the most recent available CR sample at time of screening (NOTE: will often be the sample used to diagnose AKI). eGFR should be calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula. In Japan, the CKD-EPI formula with Japanese coefficient should be used.[3] If local regulations prohibit correcting for race in the calculation of eGFR, it is acceptable to use the formula without correcting for race.
18. Not feasible to start trial drug within:
    a) 48 hours from AKI diagnosis, when AKI diagnosis precedes start of vasopressor therapy

    or

    b) 24 hours from AKI diagnosis, when AKI is diagnosed after start of vasopressor therapy.
19. Pregnant or nursing women.

Number of Patients and Sites:

A minimum of approximately 450 and a maximum of approximately 1,400 patients in the main trial population are planned to be enrolled at approximately 100-120 sites predominantly across Europe, North America, Japan, and Australia. An additional up to approximately 100 patients in the 'moderate' CKD population are planned to be enrolled at selected sites. Finally, up to approximately 100 patients in the COVID-19 population are planned to be enrolled. The final number of patients to be enrolled will depend on the recommendations of the DMC based upon the safety data reviews and interim analyses for futility/success.

Trial Drug, Dosage, and Route of Administration:

Trial drug (recAP or placebo) is provided in glass vials as a concentrate for infusion (aqueous buffer at a pH of 7.0). Prior to administration, the trial drug will be diluted with sterile sodium chloride 0.9% for injection (isotonic saline), USP/EP or equivalent, to a final volume of 50 mL and administered as an IV infusion using a dosing syringe or infusion bag. The intended recAP dose is 1.6 mg (1,000 U) per kg of patient body weight. Patients with a body weight >120 kg will be administered a fixed dose of 192 mg.

Trial drug (recAP or placebo) will be administered as a 1-hour continuous IV infusion on Day 1, Day 2 and Day 3 by qualified staff in the ICU or intermediate care unit. The first infusion is to

start as soon as feasible after randomization of the patient on Day 1. On Day 2 and Day 3, trial drug administration should start 24 +/- 2 hours after the previous trial drug administration. In case the patient has been discharged from the ICU or intermediate care unit to a ward within the hospital before completing the last trial drug administration on Day 3, trial drug should be administered at the ward by qualified personnel following instructions provided by the trial team.

The preferred route for trial drug administration is through a central catheter; if not feasible, a peripheral line is acceptable. Trial drug will be administered separately from any other concomitant drugs using a dedicated lumen of the catheter.

Statistical Methods:

Database snapshots will be used for safety data reviews and interim analyses.

A further database snapshot will be executed after all patients in the main trial population have reached Day 28. Analysis of the primary endpoint will be performed on these data by the unblinded statistician and the results, i.e., whether the primary endpoint was met, will be presented to the Sponsor in a blinded manner. No further analyses will be performed at this time.

An interim lock will take place after all patients in the main trial population have reached Day 90. Endpoints defined up to and including Day 90 will be analyzed and the results used to start the preparation of the Clinical Trial Report (CTR). All personnel involved in patient care, data collection or data monitoring will remain blinded to the individual patient's treatment allocation to minimize bias of ongoing data collection.

The final database lock (DBL) will take place after all patients have completed the trial (i.e., all patients have completed Day 180 or have withdrawn/are LTFU prior to Day 180).

If patients in the 'moderate' CKD population have not completed the trial at the time of the interim lock at Day 90 and/or final DBL at Day 180 for patients in the main trial population, a separate interim lock at Day 90 and/or final DBL at Day 180 may be performed for patients in the 'moderate' CKD population in order for the analysis of data from patients in the main trial population to commence. If the COVID-19 population completes the trial at any time prior to the final DBL, an interim lock may be performed for these patients only. Full details of interim locks and the final DBL(s) will be documented.

All statistical analyses of efficacy endpoints will be performed on the modified Intent-to-Treat (mITT) analysis set. For each population, the mITT set is defined as all patients in the population, who were randomized to a trial drug and for whom infusion of trial drug was initiated.

Analysis of the data from each population will be presented separately. Formal analyses (incl. interim analyses), hypothesis testing and descriptive analyses will be performed on data from the main trial population, whereas only descriptive statistics (including an estimate of the treatment effect, two-sided 95% confidence intervals and one-sided p-value) will be presented for the 'moderate' CKD population and for the COVID-19 population.

Primary Endpoint

The primary efficacy endpoint is "28-day all-cause mortality", defined as the probability to die (from any cause) up to and including Day 28. The primary analysis will be based on a logistic

regression model with site as a random effect, treatment as a fixed effect, mSOFA score as an ordered categorical variable, and eGFR at Baseline as the single continuous covariate.

In the event of missing survival status data, the primary analysis (including the interim analyses) will utilize multiple imputation based on a logistic regression model fitted to the group of patients with data on the primary endpoint.

The following sensitivity analyses will be conducted for the primary endpoint:

- Logistic regression as for the primary analysis with the additional covariates Acute Physiology and Chronic Health Evaluation (APACHE) II score and time from fulfilling both inclusion criteria 3 and 4 to time of treatment (hours). This analysis assesses the robustness of the findings to imbalances in those two covariates.
- Day 28 all-cause mortality obtained based on Kaplan-Meier (KM) curves for time to death up to Day 28. The KM curves will be compiled separately for the mSOFA categories (≤9 versus >9) and treatment differences in Day 28 survival rates combined using a stratified z-test. In this analysis, patients with unknown vital status who withdraw prior to Day 28 will be censored at the time of withdrawal. Patients ongoing in the trial who are known to be alive beyond Day 28 at the time of the analysis will be censored at Day 28. Patients LTFU prior to Day 28 will be censored at their last date known to be alive. This analysis assesses the impact of missing data on survival status on Day 28.
- A tipping point analysis in which all recAP patients with missing data on survival status on Day 28 will be considered as being dead while all placebo patients with missing data on survival status on Day 28 will be considered as being alive and all possible combinations of missing data between these two extremes will be considered.

Secondary Endpoints

Multiplicity for the analysis of secondary efficacy endpoints will be controlled by initiating the test procedures for secondary efficacy endpoints only if the null hypothesis for the primary efficacy endpoint has been rejected and using sequential conditional testing of null hypotheses for secondary efficacy endpoints in the order as indicated in the objectives and endpoints section; the nominal 1-sided significance level used within the sequential testing will be at the same alpha allocated to the primary endpoint at the time of the analysis.

Major Adverse Kidney Events (MAKE) 90

MAKE 90 is defined as dead by Day 90 *or* on RRT at Day 90 *or* ≥25% decline in eGFR on both Day 28 and Day 90 relative to the known or assumed pre-AKI reference level. The primary analysis will be based on a logistic regression model with site as a random effect, treatment as a fixed effect, mSOFA score as an ordered categorical variable, and pre-AKI reference eGFR as the single continuous covariate. The presentation of the results and the handling of missing data will be as described for the primary endpoint.

Days alive and free of organ support through Day 28 (with death within 28 days counting as zero days)

Days alive and free of organ support through Day 28 is to be defined as days alive with no MV, RRT, vasopressors or inotropes and with death within 28 days counting as zero days.

It is likely that these data will have distributions in each of the two treatment groups that are non-Normal and to deal with this the primary analysis will utilize a non-parametric method. The method for assessing statistical significance will be a re-randomization test comparing the treatment median values for days alive and free of organ support, respecting randomization according to site and mSOFA score. 95% confidence intervals (CIs) for the difference in the medians will be constructed to aid interpretation.

Days alive and out of the ICU through Day 28 (with death within 28 days counting as zero days)

The analysis of this endpoint will be as for days alive and free of organ support through Day 28.

Time to death through Day 90

The primary analysis will be based on the Cox proportional hazards model with site as a random effect, treatment as a fixed effect, mSOFA score as an ordered categorical variable, and eGFR at Baseline as the single continuous covariate. The treatment effect will be expressed as a hazard ratio (HR) together with a 95% two-sided CI.

Other Endpoints

Tertiary/exploratory efficacy endpoints will be summarized by trial drug group. Tertiary endpoints will be viewed as exploratory and, if applicable, a nominal 1-sided significance level of 0.025 will be used and/or 2-sided 95% CIs will be provided.

All statistical analyses of safety will be performed on the Safety Set. Incidence of all AEs, SAEs, and treatment emergent AEs (TEAEs) categorized by Medical Dictionary for Regulatory Activities (MedDRA) System Organ Class (SOC) and Preferred Term (PT) will be summarized by trial drug group. Adverse event seriousness, severity, relationship to trial drug and whether leading to discontinuation of trial drug will also be displayed in summaries and listings.

A population PK analysis of plasma concentration-time data will be performed using non-linear mixed-effects modeling. Data from this trial may be combined with data from Phase 1 in healthy adult volunteers and/or Phase 2 trial (STOP-AKI) in SA-AKI patients and included in an integrated PK analysis.

Sample Size and Power

The maximum sample size (PP_{max}) of 1,400 patients in the main trial population provides approximately 85% power assuming a 35% rate of mortality under placebo and an 8% absolute treatment effect (i.e., ~23% relative reduction assuming a 35% 28-day all-cause mortality in the placebo group). At this PP_{max}, the smallest absolute difference between active and placebo that would be declared statistically significant is approximately 5% (i.e., ~14% relative reduction). The trial's overall Type I error rate across the planned interim analyses is controlled by using the Lan-DeMets approximation of the O'Brien-Fleming alpha-spending function to determine the critical values for declaring trial success at the interim and final analyses (Table 2).

No formal sample size determination based on power calculation was performed for the 'moderate' CKD population or the COVID-19 population as no formal hypothesis testing is planned for these two populations. Up to approximately 100 patients in each population will be enrolled, which is considered adequate for the exploratory nature of these populations.

Table 2: Lan DeMets O'Brien-Fleming Nominal One-Sided *p*-values Required for Early Success at each Interim Analysis

Number of Patients Complete	Nominal One-Sided *p*-value
700	0.0015
850	0.0036
1,000	0.0067
1,400	0.0224

1.2 Trial Design and Trial Flowchart for the Individual Patient

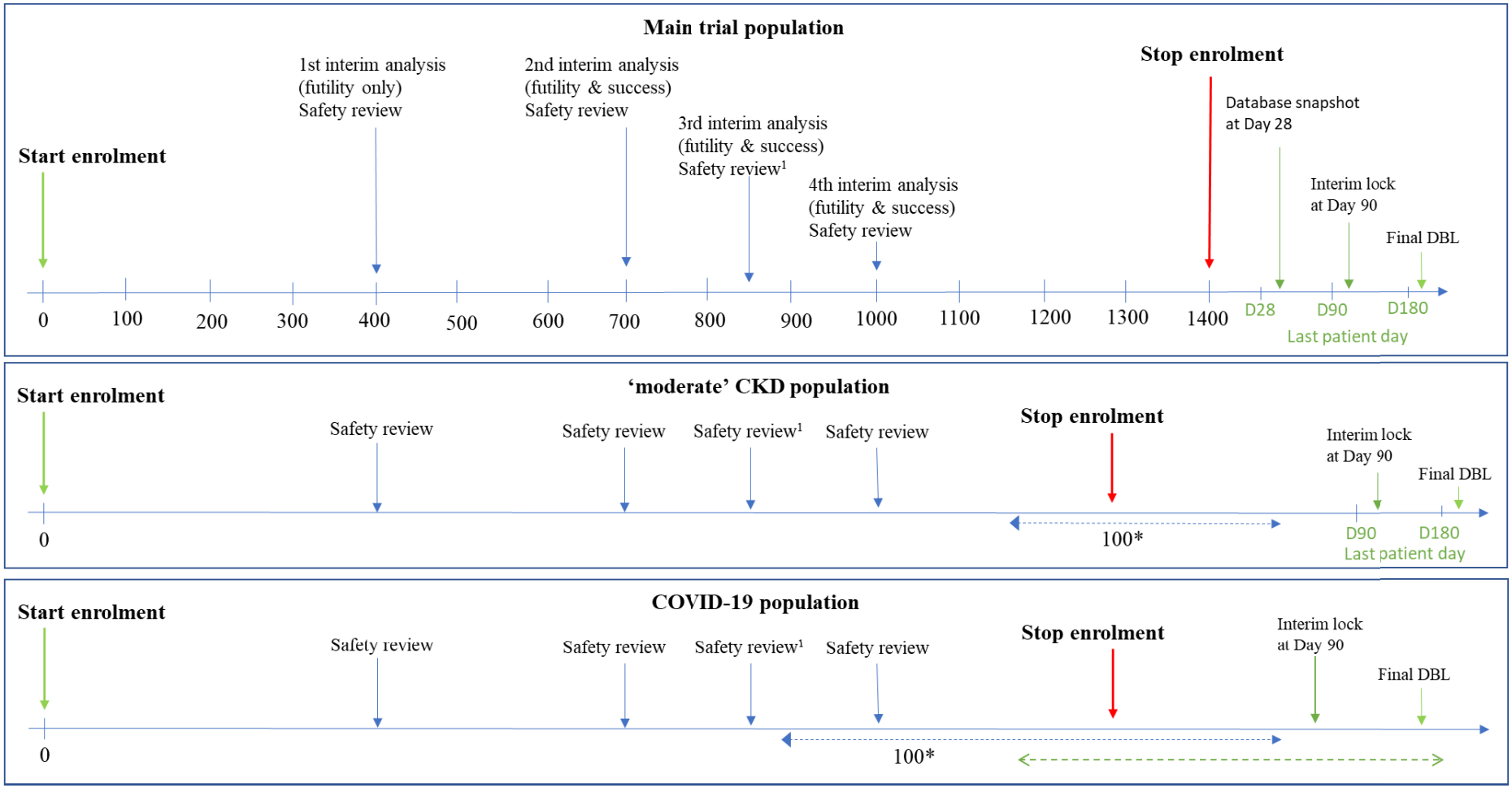

Figure 1: **Trial design**

Up to 1,400 patients in the main trial population, up to approximately 100 patients in the 'moderate' CKD population and up to approximately 100 patients in

the COVID-19 population will be enrolled. There will be a maximum of four interim analyses; after approximately 400, 700, 850, and 1,000 evaluable patients (i.e., treated patients in the main trial population who have reached Day 28), respectively. At the interim analyses, the trial may be stopped for futility or, from 700 patients onwards, for success (i.e., pre-defined p-value for primary endpoint met in the main trial population). Safety will also be assessed at regular intervals and the trial may be stopped or modified for safety concerns[1]. In the 'moderate' CKD population and in the COVID-19 population, only safety will be assessed at the interim analyses. If the trial is not stopped at one of the interim analyses, a database snapshot will be executed after 1,400 patients in the main trial population have reached Day 28 to determine if the primary endpoint was met. No further analyses will be performed at this time. An interim lock will take place after all patients in the main trial population have reached Day 90. Endpoints defined up to and including Day 90 will be analyzed and the results used to start the preparation of the CTR. The final DBL will take place after all patients have completed the trial (i.e., all patients have completed Day 180 or have withdrawn/are lost to follow-up prior to Day 180). If patients in the 'moderate' CKD population have not completed the trial at the time of the interim lock at Day 90 and/or final DBL at Day 180 for patients in the main trial population, a separate interim lock at Day 90 and/or final DBL at Day 180 may be performed for patients in the 'moderate' CKD population in order for the analysis of data in the main trial population to commence. A separate interim lock may also be performed for the COVID-19 population.

[1]: A full safety review at the time of the 850-patient interim analysis will only be performed if a futility or success threshold is reached.

Abbreviations: CKD = chronic kidney disease; CTR = Clinical Trial Report; DBL = database lock; eGFR = estimated glomerular filtration rate; ICF = informed consent form; mSOFA = Modified Sequential Organ Failure Assessment score (excluding Glasgow Coma Score); recAP = recombinant human alkaline phosphatase.

Timelines for eligibility

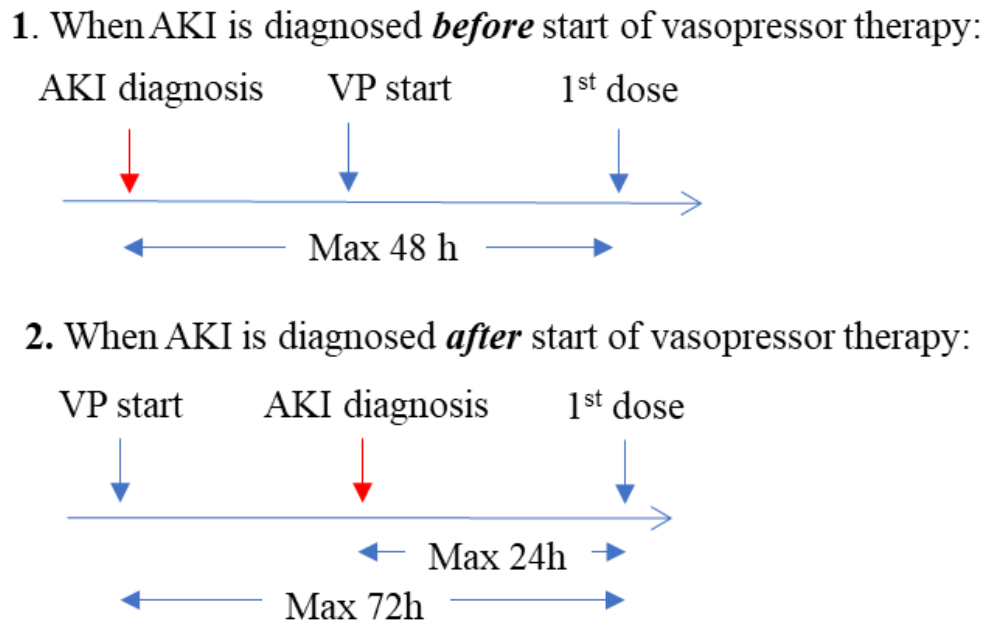

Figure 2: Timelines for Eligibility

To be eligible for the trial, patients must have both sepsis requiring vasopressor therapy and AKI. 1. When AKI is diagnosed before the start of vasopressor therapy, infusion of 1st dose of trial drug must start within 48h of AKI diagnosis. 2. When AKI is diagnosed after start of vasopressor therapy, infusion of 1st dose of trial drug must start within 24h of AKI diagnosis and no more than 72h from start of continuous vasopressor therapy for sepsis-induced hypotension. Start of AKI is defined as the timepoint where the patient for the first time meets any one of the inclusion criteria 4a)-d). Start of vasopressor therapy is defined as the start time of any dose of vasopressor in the first vasopressor treatment period that includes a continuous infusion of ≥0.1 µg/kg/min norepinephrine (or equivalent) for sepsis-induced hypotension for at least one hour in patients who have received adequate fluid resuscitation in accordance with clinical judgement and the recommendations of the Surviving Sepsis Campaign guidelines. A minimum of 12h without any vasopressor is needed to consider start of vasopressor therapy as a new episode.

Abbreviations: AKI = acute kidney injury; h = hour; recAP = recombinant human alkaline phosphatase; VP = vasopressor.

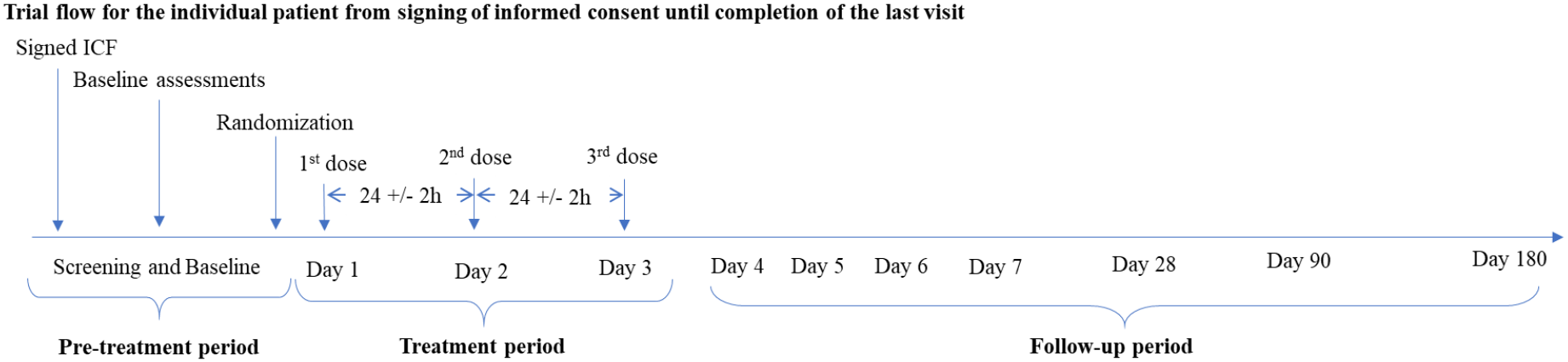

Figure 3: Trial Flow for the Individual Patient

The trial consists of a Pre-treatment Period during which Screening, Baseline assessments and Randomization will be performed, a Treatment Period (Day 1 to Day 3) during which the patient will receive a daily 1-hour continuous IV infusion of trial drug, and a Follow-up Period (Day 4 to Day 180) during which follow-up assessments will be performed.

Abbreviations: h = hour; ICF = informed consent form.

1.3 Schedule of Activities

The schedule of activities (SoA), as outlined in Table 3, consists of a Pre-treatment Period, a Treatment Period and a Follow-up Period.

Table 3: Schedule of Activities

	Pre-treatment Period		Treatment period			Follow-up period***							
Assessments	**Screening**	**Baseline***	**Day 1****	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**	**Day 28 (+4 days)**	**Day 90 (+10 days)**	**Day 180 (+10 days)**	**EOT (in case of withdrawal)**
Eligibility check (inclusion/exclusion criteria)	X												
Informed consent	X												
Pregnancy test (urine or blood), WOCBP only[6]	X												
Serum/plasma CR[1]		X	Daily until ICU or intermediate care unit discharge (max to Day 7)							X	X		
KDIGO AKI Stage		X	Daily until ICU or intermediate care unit discharge (max to Day 7)							X	X		
Medical history[2]		X											
Demographics		X											
Weight, height[3]		X											
12-lead ECG		X											
mSOFA score[4]		X	Daily until ICU or intermediate care unit discharge (max to Day 7)										
APACHE II (incl. GCS)		X											
Main cause of sepsis[5]		X											
Hematology[7]		X			X					X (or at hospital discharge if before Day 28)			X[8]
Clinical chemistry[7]		X			X					X (or at hospital discharge if before Day 28)			X[8]
Blood sampling Biomarkers[9]		X	X	X	X	X	X			X			
Blood sample for ADA[9,10]		X								X	X		X
Randomization[11]		X											
Trial drug administration[12]			X	X	X								
PK samples[13,9]					X	X	X		X				

	Pre-treatment Period		Treatment period			Follow-up period***							
Assessments	**Screening**	**Baseline***	**Day 1****	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**	**Day 28 (+4 days)**	**Day 90 (+10 days)**	**Day 180 (+10 days)**	**EOT (in case of withdrawal)**
Urine samples for purine determination[14]		X	X	X	X	X							
Functional Status and Residency[15]		X								X	X	X	
QoL EQ-5D-5L[16]		X								X	X	X	
Mechanical ventilation [17]		X											
Vasopressor/ Inotropic use[18]		X											
Renal replacement therapy[19]		X									X		
Prior and concomitant medication[20]		X (until hospital discharge)											
AE[21]		X											
ICU/hospital admission /discharge[22]		X											
Rehospitalization[23]		X											
Mortality[24]		X											

Abbreviations: ADA = anti-drug antibodies; AE = adverse events; AP = alkaline phosphatase; APACHE II = Acute Physiology and Chronic Health Evaluation II; CR = creatinine; ECG = electrocardiogram; eCRF = electronic case report forms; EOT = end of trial; GCS: Glasgow Coma Scale; ICU = intensive care unit; KDIGO AKI = Kidney Disease Improving Global Outcomes acute kidney injury; mSOFA = modified Sequential Organ Failure Assessment; MV = mechanical ventilation; PK = pharmacokinetic; QoL EQ-5D-5L = Quality of Life EuroQoL-5-Dimensions-5 Levels; SA-AKI = sepsis-associated acute kidney injury; RRT = renal replacement therapy; SAE = serious adverse event; SOFA = Sequential Organ Failure Assessment; WOCBP = women of child bearing potential.

* Assessments performed for clinical purposes before start of infusion of trial drug may be used as Baseline if deemed appropriate.

** If Day 1 and Baseline occur on the same calendar day, there is no requirement to do trial specific assessments on Day 1 after trial drug administration. Results of assessments done for clinical purposes on Day 1 after trial drug administration should be entered in the eCRF.

*** The follow-up assessments may be performed in the hospital for patients still in the hospital or able to come to the hospital after discharge or at home if the patient is not able to attend after discharge. Home visits can be performed by trial personnel, if allowed by local regulations, or by a third-party vendor.

[1] Performed by local laboratory. If multiple CR values are available on the day of Baseline, the value closest to the time of randomization should be recorded. If Day 1 and Baseline is on the same calendar day, there is no requirement to repeat measurements on Day 1 after trial drug administration. From Day 2 onwards, if more than one CR value is available on a single day, the worst daily value should be recorded.

[2] Medical history will be collected by using the Charlson co-morbidity index supplemented with recent medical history of relevance to this episode of SA-AKI including whether the reason for ICU admission is medical, surgical or trauma (see Section 8.3.2.2).

[3] Known hospital admission weight or estimated weight and height can be used. Weight in kg will be used for trial drug reconstitution.

[4] mSOFA (i.e., excluding the Glasgow Coma Score) is to be obtained daily from Baseline to Day 7 (see Section 8.3.3.4). On days where hematology and clinical chemistry measurements are required as part of safety measurements, these results can be used for the mSOFA score. On days where hematology and clinical chemistry measurements are not required, the platelets, bilirubin, and CR must be measured by the local laboratory to calculate a mSOFA score. If the patient is discharged from the ICU or intermediate care unit before Day 7, mSOFA need not be collected. At Baseline, mSOFA must be available before randomization for stratification. If Day 1 and Baseline is on the same calendar day, mSOFA does not need to be obtained again on Day 1 after trial drug administration.

[5] Record, when available. See Section 8.3.2.8 for specific information to be recorded.

[6] Performed by local laboratory. Both urine and blood pregnancy tests are allowed. Only to be performed for WOCBP.

[7] Performed by local laboratory, see Section 8.3.4.2. AP activity should not be measured during the first 14 days after the first trial drug administration or results from blood samples taken during the first 14 days of the trial are not to be reported to trial team members or to any other blinded personnel involved in patient care and/or data collection as it could lead to unblinding and to erroneous interpretation of liver function, as the recAP administered will increase the AP activity. Blinding plans will be made and approved for each site before the start of the trial and must be followed (Section 6.3.2).

[8] Only to be performed if patient withdraws before Day 28 (inclusive).

[9] Handling, storing and shipment of the samples is described in the Laboratory Manual (Section 8.3.3.11).

[10] ADA Samples taken on Day 90 will only be analyzed in case of a positive result on Day 28. At Day 180 and Day 360, a blood sample for ADA will only be taken in patients with a positive ADA response on Day 90 and Day 180, respectively. Collection of ADA sample for patients withdrawn are only required if EOT visit takes place after Day 28 and before Day 90. Handling, storing and shipment of the samples is described in the Laboratory Manual (Section 8.3.4.3).

[11] mSOFA score must be available for the stratification at randomization.

[12] On Day 2 and Day 3, trial drug administration should start 24 +/- 2 hours after the previous trial drug administration.

[13] On Day 3, a PK sample must be collected before trial drug administration and another sample 2.5 - 3.5 hours after the start of the infusion. On Day 4, Day 5, and Day 7, PK samples can be taken at any time (Section 8.3.5.1). The exact date and time of blood draw must be recorded in the eCRF.

[14] In patients at selected sites, a daily urine sample will be collected at Baseline up to Day 4 (Section 8.3.3.11). At the dosing days (Day 1, Day 2 and Day 3), the urine sample must be taken within one hour after the end of trial drug administration. On Day 4, urine collection can be performed at any time. Handling, storing and shipment of the samples is described in the Laboratory Manual.

[15] Functional status and residence at Baseline will be a recall of the patient's situation prior to hospitalization for SA-AKI (Section 8.3.3.9).

[16] Baseline refers to a recall of the patient's quality of life before the current SA-AKI episode. If a patient is not able to perform the QoL at Baseline (e.g., due to sedation), the patient should complete the baseline QoL when his/her medical condition allows it. If a patient is unable to complete the EQ-5D-5L questionnaires themselves, the questionnaire can be completed by an interview by reading the questions and answers objectively. If the patient is discharged from hospital, the questionnaire may be completed by a phone interview (Section 8.3.3.8).

[17] Mechanical ventilation (MV) is defined as any positive pressure ventilation via endotracheal or tracheostomy tube or any non-invasive ventilation with >5 cm H_2O pressure (see Section 8.3.3.5). Record MV start and stop dates together with information on the modality/modalities.

[18] Record the start and stop time and date for each inotropic and vasopressor drug. For additional information to be recorded, see Section 8.3.3.7.

[19] All renal replacement therapy (RRT) modalities (continuous, intermittent and non-continuous) are allowed in the trial. Up to Day 28, record the main reason to start RRT, the modality, and start and stop date in the eCRF. At Day 90, record the RRT status (Section 8.3.3.6).

[20] Relevant prior and concomitant medication should be collected up to and including Day 28 or until hospital discharge if before Day 28. Concomitant medications/procedures that are likely to influence the outcome of the SA-AKI and nephrotoxic drug have to be detailed. See Section 6.5 for more details.

[21] Safety is followed up until Day 28 (inclusive). However, ongoing SAE's on Day 28 will be followed until resolution or until they have reached a stable medical condition (Section 8.3.4.4.1).

[22] ICU or intermediate care unit and hospital admission and discharge dates as well as reason for admissions must be recorded (Section 8.3.3.10).

[23] Rehospitalization is defined as an overnight stay in any hospital (Section 8.3.3.10).

[24] Record date and cause of death on a separate eCRF page. Cause of any death occurring up to and including Day 28 will additionally be recorded as an SAE (Section 8.3.3.1).

2 Introduction

Recombinant human alkaline phosphatase (recAP) is a full-length human chimeric alkaline phosphatase (AP) that is being developed for the treatment of sepsis-associated acute kidney injury (SA-AKI).

2.1 Trial Rationale

Sepsis is the leading cause of acute kidney injury (AKI) and a major cause of death. Patients with SA-AKI have a high mortality and morbidity and are at risk of developing chronic kidney disease (CKD). AP is a homodimeric endogenous enzyme present in many cells and organs, e.g., intestines, placenta, liver, bone, kidney, and granulocytes. It exerts detoxifying effects through dephosphorylation of endotoxins; pathogen-associated molecular pattern molecules (PAMPS e.g., lipopolysaccharide [LPS]) and damage-associated molecular pattern molecules (DAMPS e.g., adenosine tri- and di-phosphate [ATP and ADP]). In animal models of sepsis and AKI, administration of AP attenuates the inflammatory response, improves renal function and/or reduces mortality.

AM-Pharma B.V. (hereafter referred to as the Sponsor) is developing AP as a novel, recombinant chimeric human AP medicinal product, called recAP, to be used as an intravenous (IV) infusion for the treatment of SA-AKI. In the Phase 2 trial STOP-AKI, a survival benefit was observed in the two highest dose groups, 0.8 mg/kg and 1.6 mg/kg groups, compared to the placebo group. There were no safety or tolerability concerns for any of the doses tested (0.4, 0.8 and 1.6 mg/kg). The 1.6 mg/kg recAP dose was selected for this Phase 3 trial based on the significant survival benefit observed. Pharmacokinetic/pharmacodynamic (PK/PD) simulations also confirmed this dose to have the most pronounced treatment effect.

The primary objective of this Phase 3 trial is to confirm the mortality benefit seen in STOP-AKI by demonstrating a reduction in 28-day all-cause mortality in patients with SA-AKI treated with 1.6 mg/kg recAP.

Refer to Section 4.2 for the scientific rationale for the trial design.

2.2 Background

2.2.1 Sepsis and Acute Kidney Injury

Sepsis is a pathophysiological dysregulated host response to infection causing organ dysfunction. It is estimated that around 19 million people each year are affected by sepsis[4]. The increased awareness through the Surviving Sepsis Campaign and new sepsis diagnosis criteria (Sepsis-3[5]), has focused on prompt identification of sepsis, early interventions and improved critical care, resulting in a better reporting and estimation of sepsis incidence[6, 7, 8, 9, 10]. Due to the significant increase of infections with multidrug resistant pathogens during recent years, mortality in the intensive care units (ICUs) due to sepsis and septic shock remains high with a mortality rate between 35% and 40%[11]. Thus, sepsis is the primary cause of death in surgical ICUs and the third cause in non-surgical ICUs[12].

Sepsis is the leading cause of AKI, a serious medical condition with an in-hospital mortality rate of approximately 40%[13, 14, 15]. Patients who survive a SA-AKI episode are at risk of developing

CKD, resulting in a high burden for the patient and society[16, 17, 18]. AKI is a multi-factorial condition with inflammatory, direct nephrotoxic, and ischemic insults acting simultaneously with other pathogenic responses to rapidly cause dysfunction or failure of the kidney[19, 20, 21, 22].

In sepsis, the initial host response to an infection, mostly caused by bacteria, becomes amplified and dysregulated, bringing the body in an overall inflammatory state[23]. Currently, there are no pharmacological interventions approved for the treatment of SA-AKI, and RRT is the only supportive treatment option available for these patients[13, 15, 24].

2.2.2 Alkaline Phosphatase

Alkaline phosphatase is an endogenous homodimeric enzyme present in many cells and organs (e.g., intestines, placenta, liver, bone, kidney, and granulocytes) that exerts detoxifying effects through dephosphorylation of endotoxins[25, 26] and other pro-inflammatory compounds, including extracellular ATP[27] by hydrolysis. Local AP concentrations reflect the host defense against endotoxin in the kidney[28]. During ischemia, AP levels are markedly depleted, associated with the development of AKI[29]. Apart from local effects in the kidney, AP may also attenuate the innate immune response, as dephosphorylation of endotoxin abolishes its biological activity and the dephosphorylated LPS acts as a toll-like receptor 4 (TLR4) antagonist[30]. In animal models of sepsis, AP administration attenuates the inflammatory response and reduces mortality[31, 32]. There is increasing evidence that AP plays a significant role in host defense and innate immunity, particularly against inflammatory reactions due to LPS release[31, 33].

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[34] and are released when these bacteria divide or disintegrate. It is a group of negatively charged molecules, of which the Lipid A moiety binds to the TLR4-MD-2 complex through two, for this purpose essential, phosphate groups. A hexameric complex composed of two copies of the TLR4–MD-2–LPS complex is essential for signaling[35]. The receptors are present on the surface of leukocytes (macrophages, white blood cells) and endothelial cells. Once activated, these cells secrete a number of inflammatory cytokines that can cause a devastating and life-threatening derailment of the human innate immune system.

The mode of action of AP is assumed to be dual:

- First, it binds, and subsequently dephosphorylates the PAMP, LPS[36], thereby eliminating and blocking the root cause of the systemic inflammatory responses. 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 systemic inflammation, and prevent progression into sepsis and septic shock. Furthermore, in ongoing sepsis, it attenuates the peaks of the inflammatory response induced by LPS entering the systemic circulation.
- Second, AP acts by dephosphorylating DAMPS, e.g., the pro-inflammatory extracellular ATP via ADP and adenosine monophosphate (AMP) to the anti-inflammatory and tissue protective adenosine[36, 37]. As a result, dephosphorylation by AP reduces the inflammatory responses and provides an anti-inflammatory effect[38, 39, 40].

2.2.3 Recombinant Human Alkaline Phosphatase (recAP)

RecAP is a full-length human chimeric AP derived by recombinant technology and produced in Chinese hamster ovary cells. RecAP 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. The glycosylphosphatidylinositol (GPI) anchor has been removed resulting in a secretable form. RecAP has a projected mass of approximately 105 kDa based on the amino acid sequence derived from the deoxyribonucleic acid (DNA) sequence and approximately 130 kDa as a fully glycosylated molecule.

2.2.4 Pre-clinical and Clinical Studies with Recombinant Human Alkaline Phosphatase (recAP)

Pre-clinical studies with recAP in rodent and porcine ischemia-reperfusion or systemic endotoxemia models showed that a single dose of recAP consistently inhibited markers of inflammation and reduced kidney damage and functional impairment as assessed by serum creatinine (SCR), blood nitrogen, creatinine clearance and histopathological examination. Ischemia-reperfusion experiments with a CD73-/-knockout mouse (not able to produce extracellular adenosine) showed that recAP had protective effects through the generation of tissue-protective adenosine, which acts on the A2A adenosine receptor.

In a Phase 1 single ascending dose (SAD) and multiple ascending dose (MAD) clinical trial in 51 healthy volunteers, all reported adverse events (AEs) were classified as mild or moderate and most were evenly distributed over the active and the placebo groups. The trial did not raise any safety concerns and single IV infusion of recAP in the range of 200 to 2,000 U/kg, as well as daily IV infusions of recAP at doses of 500 and 1,000 U/kg for three consecutive days, were well-tolerated by healthy male and female subjects.

A Phase 2a/2b Proof-of-Concept and Dose-Finding trial (STOP-AKI) with recAP in 301 patients with SA-AKI was recently finalized. In Part 1 of STOP-AKI, patients were randomized to receive 0.4, 0.8, or 1.6 mg/kg of recAP or placebo, once a day for three consecutive days to identify the optimal dose. At the interim analysis, 1.6 mg/kg recAP was selected by the Data Monitoring Committee (DMC) as the optimal recAP dose for Part 2. In Part 2, 1.6 mg/kg recAP was compared to placebo. The primary endpoint, the mean daily endogenous creatinine clearance (ECC) on Days 1 – 7 (area under the curve [AUC] 1-7 ECC), was not significant. However, recAP showed a significant relative reduction in 28-day mortality of more than 40%. A positive effect on mortality was also seen in the 0.8 mg/kg dose. Overall, recAP showed a long-term effect on the kidney function with significant improvement of ECC on Day 21 and Day 28 compared to Day 1. Non-fatal serious adverse events (SAEs) were comparable in the recAP and placebo groups, indicating that the drug is well-tolerated and safe.

Justification for the dose selected in this Phase 3 trial is detailed in Section 4.3. A more detailed description of recAP and results from non-clinical studies are provided in the Investigator Brochure (IB).

2.3 Benefit/Risk Assessment

Alkaline Phosphatase is a common endogenous enzyme present in many cells and organs (e.g., intestines, placenta, liver, bone, kidney and granulocytes). A recombinant human version of alkaline phosphatase (recAP) is being developed for the treatment of SA-AKI.

Phase 1

A total of 51 male and female healthy volunteers were included in the Phase 1 SAD and MAD trial[41], of which 37 subjects were exposed to single doses of up to 2,000 U/kg (3.2 mg/kg) recAP or daily recAP doses of 500 U/kg (0.8 mg/kg) or 1,000 U/kg (1.6 mg/kg) administered for three consecutive days. No SAEs were observed and there were no clinically significant findings from clinical laboratory, 12-lead electrocardiogram (ECG), continuous cardiac monitoring or physical examination during the trial. All treatment emergent AEs (TEAEs) were transient and had resolved without sequelae by the follow-up period. None of the subjects were positive for anti-drug antibodies (ADAs). The overall percentage of subjects reporting TEAEs was generally comparable in the placebo and recAP groups. Furthermore, the number of TEAEs did not increase with increasing dose.

In summary, single IV infusions of recAP in the range of 200 U/kg (0.32 mg/kg) to 2,000 U/kg (3.2 mg/kg), as well as daily IV infusions of recAP at doses of 500 U/kg (0.8 mg/kg) or 1,000 U/kg (1.6 mg/kg) administered for three consecutive days were well-tolerated by healthy male and female subjects.

Phase 2

In the Phase 2 trial (STOP-AKI)[42, 43], patients with SA-AKI were treated with three doses of recAP (0.4, 0.8 and 1.6 mg/kg) or placebo. A total of 182 patients with SA-AKI were exposed to recAP.

One patient in the 0.4 mg/kg group discontinued from the trial due to a non-serious TEAE of severe hypoxia. The patient was a 49-year-old Caucasian male with SA-AKI who was randomly assigned to the recAP 0.4 mg/kg (250 U/kg) treatment group. The event of hypoxia was considered not recovered/not resolved when the patient discontinued from the trial. The Investigator assessed the event of hypoxia to be unrelated to the trial drug. In general, hypoxia was observed incidentally with no difference between dosing groups.

Serious TEAEs were reported in 43.1% and 50.0% of patients in the 1.6 mg/kg recAP and placebo groups, respectively, reporting a total of 76 and 89 serious TEAEs, respectively. The occurrence of non-serious TEAEs was comparable between the 1.6 mg/kg recAP and placebo groups and the majority of TEAEs were considered by the Investigator to be unrelated to trial drug. The percentage of TEAEs considered by the Investigator to be possibly related to trial drug was 20.2% and 13.4% in the 1.6 mg/kg recAP group and placebo group, respectively. Most TEAEs were mild or moderate in severity. Fatal SAEs were reported in 26.3% of the patients in the 0.4 mg/kg recAP group, 17.1% of the patients in the 0.8 mg/kg recAP group, 17.4% of the patients in the 1.6 mg/kg recAP group and 29.5% of patients in the placebo group, indicating a potential benefit on survival.

Post-hoc analyses of ECC resulted in a higher ECC in the treatment group compared to placebo (mean difference of 27.6 mL/minute for Day 21 and a mean difference of 18.2 mL/minute for Day 28). These improvements in ECC at 1.6 mg/kg in the recAP group compared to the placebo group indicated a potential benefit on long-term kidney function.

The two clinical studies conducted to date with recAP showed that recAP is safe and well-tolerated with potential clinical benefits observed in the Phase 2 trial (STOP-AKI).

In the current Phase 3 trial, patients with SA-AKI will be randomly assigned to receive either 1.6 mg/kg recAP or matching placebo as a 1-hour IV infusion on three consecutive days. Trial drug is given as add-on to standard of care for sepsis and AKI patients as defined in the most recent Surviving Sepsis Campaign guidelines[5] and the Kidney Disease Improving Global Outcomes [KDIGO] Clinical Practice Guidelines for Acute Kidney Injury[44]. Currently, no specific treatment is available for SA-AKI, therefore placebo is the relevant comparator.

To date, a total of 219 patients and healthy volunteers have been exposed to recAP. The risk profiles from both the Phase 1 and the Phase 2 studies were favorable. However, the experience in SA-AKI patients treated with recAP is limited and the occurrence of AEs with relatively low incidence cannot be excluded.

In conclusion, the favorable safety and tolerability profile observed in the Phase 1 and Phase 2 studies in combination with the marked clinical benefits on survival and improved long-term kidney function observed in the Phase 2 trial justify a confirmatory Phase 3 trial using the same dose regimen of 1.6 mg/kg recAP as in the Phase 2 trial (STOP-AKI).

3 Objectives and Endpoints

The following objectives and endpoints are defined for the main trial population.

The same objectives and endpoints will also be assessed and explored in the 'moderate' CKD population and the COVID-19 population without any formal hypothesis testing.

OBJECTIVES	ENDPOINTS
Primary	
To demonstrate an effect of recAP on 28-day all-cause mortality.	28-day all-cause mortality.
Secondary	
To investigate the effect of recAP on long-term Major Adverse Kidney Events (MAKE).	MAKE 90: dead by Day 90 *or* on RRT at Day 90 *or* ≥25% decline in estimated glomerular filtration rate (eGFR) on both Day 28 and Day 90 relative to the known or assumed pre-AKI reference level.
To investigate the effect of recAP on use of organ support, i.e., mechanical ventilation (MV), Renal Replacement Therapy (RRT), vasopressors or inotropes.	Days alive and free of organ support through Day 28, i.e., days alive with no MV, RRT, vasopressors or inotropes (with death within 28 days counting as zero days).
To investigate the effect of recAP on length of stay (LOS) in ICU.	Days alive and out of the ICU through Day 28 (with death within 28 days counting as zero days).
To investigate the effect of recAP on 90-day all-cause mortality.	Time to death through Day 90.
Tertiary/Exploratory	
To investigate the effect of recAP on 180-day all-cause mortality.	Time to death through Day 180.
To investigate the effect of recAP on organ function in the first week.	Change in total and individual organ failure scores through Day 7 (based on the modified Sequential Organ Failure Assessment [mSOFA] scores defined as the SOFA score without the Glasgow Coma Scale [GCS] component).
To investigate the effect of recAP on short and long-term renal function.	Days alive and free of RRT through Day 28 (with death within 28 days counting as zero days). MAKE 28: dead by Day 28 *or* on RRT at Day 28 *or* ≥25% decline in eGFR on both Day 7/ICU discharge (whichever comes

OBJECTIVES	ENDPOINTS
	first) and Day 28 relative to the known or assumed pre-AKI reference level. Patients alive and free of AKI on Day 7/ICU discharge (whichever comes first) and on Day 28. Patients alive and free of new onset CKD or worsening of CKD (defined as any increase in CKD Stage) on Day 90.
To investigate the effects of recAP on cardiovascular dysfunction.	Days alive and free of vasopressor and inotropes through Day 28 (with death within 28 days counting as zero days).
To investigate the effect of recAP on pulmonary function	Days alive and free of MV through Day 28 (with death within 28 days counting as zero days).
To investigate the effect of recAP on LOS in hospital and rehospitalization.	Days alive and out of the hospital through Day 90 (with death within 28 days counting as zero days). Incidence of at least one rehospitalization at any hospital through Day 90.
To investigate the effect of recAP on Quality of Life (QoL).	Change in index values, quality-adjusted life years (QALY) and visual analogue scale (VAS) score based on the EuroQoL-5-Dimention-5 Levels (EQ-5D-5L) questionnaire at Day 28, Day 90 and Day 180.
To investigate the effects of recAP on urinary excretion of purines.	The urinary levels of purines (ATP, ADP, AMP, cyclic adenosine monophosphate [cAMP], and adenosine) through Day 4 at selected sites.
Pharmacokinetics	
To investigate the pharmacokinetic (PK) properties of recAP.	Population PK.
Safety	
To investigate the safety and tolerability of recAP.	Generation of anti-recAP antibodies on Day 28 and Day 90. Incidence of AEs and SAEs through Day 28.

4 Trial Design

4.1 Overall Design

This is a Phase 3, multi-center, randomized, double-blind, placebo-controlled, 2-arm parallel-group-sequential design pivotal trial in which patients with SA-AKI are randomly assigned in a 1:1 ratio to either placebo or 1.6 mg/kg recAP. Randomization will be stratified by:

- 'Moderate' CKD defined as a pre-AKI reference eGFR ≥25 and <45 mL/min/1.73 m^2
  - Yes
  - No
- Baseline mSOFA score, i.e., excluding the GCS part.
  - mSOFA score ≤9
  - mSOFA score >9
- Clinical site

An additional cohort of patients with SA-AKI and proven or suspected SARS-CoV-2 will be included in the trial. This cohort will also be randomly assigned in a 1:1 ratio to either placebo or to 1.6 mg/kg recAP. Randomization for this cohort will be stratified by:

- Baseline mSOFA score, i.e., excluding the GCS part.
  - mSOFA score ≤9
  - mSOFA score >9
- Clinical site

Based on this, we define three distinct SA-AKI trial populations:

1. **The main trial population:** Patients with a pre-AKI reference eGFR ≥45 mL/min/1.73 m^2 and no proven or suspected SARS-CoV-2 at time of randomization.
2. **A 'moderate' CKD population:** Patients with a pre-AKI reference eGFR ≥25 and <45 mL/min/1.73 m^2 and no proven or suspected SARS-CoV-2 at time of randomization.
3. **A COVID-19 population:** Patients with proven or suspected SARS-CoV-2 at time of randomization with or without 'moderate' CKD. For patients in this population, COVID-19 should be the main cause of SA-AKI.

Analysis of data from each population will be performed and presented separately. Formal analyses (incl. interim analyses), hypothesis testing and descriptive analyses will be performed on data from the main trial population, whereas only descriptive statistics (including an estimate of the treatment effect, two-sided 95% confidence intervals and one-sided p-value) will be presented for the 'moderate' CKD population and the COVID-19 population. Additional exploratory analyses for each of the three populations as well as for the pooled main trial population plus 'moderate' CKD population, and the pooled main trial population plus COVID-19 population are planned and will be described in the SAP.

A minimum of approximately 450 and a maximum of approximately 1,400 patients in the main trial population are planned to be enrolled at approximately 100-120 sites predominantly across Europe, North America, Japan, and Australia. An additional up to approximately 100 patients in

the 'moderate' CKD population are planned to be enrolled at selected sites. Finally, up to approximately 100 patients in the COVID-19 population are planned to be enrolled. The final number of patients to be enrolled will depend on the recommendations of the DMC based upon the safety data reviews and interim analyses for futility/success.

Participating sites should follow the internationally accepted standard of care guidelines for sepsis (Survival Sepsis Campaign 2016)[5], and those for AKI (KDIGO 2012 Clinical Practice Guidelines for Acute Kidney Injury[44]). If new versions of these guidelines are published during the trial, these should be adopted.

There will be a maximum of four interim analyses during the trial and enrolment continues during the interim analysis. The interim analyses will take place after approximately 400, 700, 850, and 1,000 evaluable patients (i.e., treated patients in the main trial population who have reached Day 28). At the first interim analysis, the trial may be stopped for futility. At subsequent interim analyses, the trial may be stopped for futility, or for success (i.e., early demonstration of superiority of recAP over placebo on 28-day all-cause mortality).

An independent DMC will evaluate safety data at regular intervals throughout the trial and notify the Sponsor and the TSC in case of safety concerns that lead to a recommendation to stop or modify the trial. Data from patients in the 'moderate' CKD population and the COVID-19 population will also be included at each safety review.

The DMC will review the interim analysis reports and notify the Sponsor and the TSC in case a futility or success threshold is reached. The DMC will follow an agreed charter and will provide written communication to Sponsor and the TSC on its recommendation on trial continuation, discontinuation or modifications based on safety data and the interim analysis reports. More details of the safety data reviews and the interim analyses will be provided in the DMC charter and DMC Statistical Analysis Plan (SAP), which will both be finalized before the first safety data review.

The duration of the trial for a patient is approximately 6 months, i.e., from the date that informed consent is provided until the last follow-up visit on Day 180. However, if a patient has a positive ADA response on Day 180, the patient will have an additional blood sample drawn at Day 360 (+/- 30 days). This is expected to only apply to a very limited number of patients and therefore will be reported separately outside the electronic case report form (eCRF).

The trial includes a Pre-treatment Period, during which the patient will be screened, baseline assessments will be performed and the patient will be randomized, a Treatment Period from Day 1 to Day 3, during which the patient will receive a daily one hour continuous IV infusion of 50 mL trial drug (recAP 1.6 mg/kg or placebo) and a Follow-up Period from Day 4 to Day 180, during which trial specific assessments and data collection will be performed. Trial drug will be provided as add-on therapy in addition to normal standard of care. Calendar days are used throughout the trial with the start of the first infusion of trial drug defining Day 1.

Safety is followed up until Day 28 (inclusive). However, SAEs ongoing on Day 28 will be followed up 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 the patient is lost to follow-up (LTFU). All death up to Day 180 will be recorded on the Mortality page of the eCRF. All SAEs that occur after Day 28 will be reported to the Sponsor or designee only if the Investigator considers them possibly, probably, or definitely related to the trial drug.

The trial design is presented in Figure 1, the timelines for eligibility in Figure 2, the trial flow for the individual patient in Figure 3, and the schedule of activities (SoA) is presented in Table 3.

4.2 Scientific Rationale for Trial Design

The favorable safety profile observed in the Phase 1 and Phase 2 studies and the significant effect on mortality observed in the Phase 2 trial (STOP-AKI) require confirmation in a larger placebo-controlled, multi-center trial.

The main trial target patient population, i.e., SA-AKI, and treatment schedule of this trial, i.e., a 1-hour IV infusion over three consecutive days, is similar to that of the Phase 2 trial (STOP-AKI). 28-day all-cause mortality is chosen as the primary endpoint in accordance with the European Medicines Agency (EMA) 2006 sepsis guidelines and is also the preferred endpoint by the United States (US) Food and Drug Administration (FDA). Mortality is an objective, clinically relevant and patient-centric outcome that incorporates both efficacy and safety.

Compared to STOP-AKI, minor changes to the eligibility criteria have been implemented (see Section 5). Most importantly, in this trial, the diagnosis of sepsis will be made based on the current Sepsis-3 criteria published in 2016[5]. The Sepsis-3 criteria are now widely used to diagnose sepsis and septic shock, instead of the sepsis-2 definition that was used in STOP-AKI, which was based on the systemic inflammatory response syndrome (SIRS) criteria. According to the Sepsis-3 criteria, sepsis is defined as life-threatening organ dysfunction caused by a dysregulated host response to infection. For clinical purposes, organ dysfunction is defined as an increase in the SOFA score of 2 points or more.

Also, as 90% of patients in STOP-AKI received vasopressor treatment, the requirement for vasopressor treatment has been implemented as an inclusion criterion. Importantly, the requirement for vasopressor therapy does not mean that the target patient population must have septic shock, as septic shock, in this protocol, is defined in accordance with the Sepsis-3 definition and clinically recognized by a vasopressor requirement to maintain a mean arterial pressure of 65 mm Hg or greater ***and*** a serum lactate level greater than 2 mmol/L (>18 mg/dL) in the absence of hypovolemia. As an elevated lactate level is not a requirement for enrolment in this trial, the target indication for this trial remains SA-AKI and not septic shock-associated AKI although patients in septic shock may be eligible as well.

In addition, we allow for enrollment of a small sample of patients with more severe CKD (i.e., the 'moderate' CKD population) than what was studied in STOP-AKI. The rationale for including this sample is that CKD patients are more prone to SA-AKI and therefore it is relevant to explore the safety and effects of recAP in these patients. However, as this is an exploratory population that was not studied in STOP-AKI, no formal analyses or hypothesis testing will be performed.

Finally, at the time of completion of this protocol, there is a global outbreak of SARS-CoV-2 causing COVID-19. As a significant number of these patients develop AKI, which may be driven by the same pathophysiological mechanisms as in SA-AKI of other causes, it is relevant to explore whether the effect of recAP is similar in these patients. As there is currently limited knowledge about AKI and associated outcomes in these patients, they are excluded from formal analyses or hypothesis testing and are only intended to provide exploratory data of the effect of recAP in COVID-19 patients. Also, the prevalence of COVID-19 during the planned trial period is unknown so there is no requirement for a minimum sample size.

Recombinant human AP is considered as an add-on to standard therapy; hence, the use of placebo for comparison is fully justified. The double-blinded design with the use of a placebo group enables unbiased evaluation of efficacy and safety in comparison to standard of care.

For this Phase 3 trial, the 1.6 mg/kg recAP dose (highest dose tested in STOP-AKI) was selected.

The design of this trial involves several interim analyses of the primary endpoint. Multiplicity introduced by repeated analyses is addressed by using the Lan-DeMets approximation of the O'Brien-Fleming alpha spending function to determine the critical values for declaring trial success at interim and final analyses.

Follow-up of patients for a period of 180 days allows for assessing potential disease-modifying effects of recAP on kidney function and investigating the long-term effect of recAP on mortality and other clinically relevant, patient-centric and health-economic outcomes. In addition, PK and ADA data will be obtained in a geographically broader and more diverse ethnic population compared to in STOP-AKI.

4.3 Justification for Dose

In the Phase 2 clinical trial STOP-AKI, a clinically relevant survival benefit was observed in the 0.8 mg/kg and 1.6 mg/kg treatment groups compared to the placebo group and there were no safety concerns for any of the doses tested (0.4 mg/kg, 0.8 mg/kg, and 1.6 mg/kg).

A PK-PD model was constructed on data from STOP-AKI, including multiple recAP doses (0.4 mg/kg, 0.8 mg/kg, and 1.6 mg/kg), recAP exposure and ECC. Simulations showed a clear dose-response on the rate of recovery in terms of ECC, with the largest benefit for the 1.6 mg/kg dose group. This treatment effect was most pronounced in the group of patients who had a low initial ECC.

Based on the effects observed in STOP-AKI in terms of survival and ECC and on the PK-PD model, which indicated that no additional treatment effect was to be expected from higher or longer dosing, the 1.6 mg/kg recAP dose was selected for this Phase 3 trial.

4.4 End of Trial Definition

The end of trial (EOT) is defined as the date on which the last patient completes the Day 180 assessments. All efforts should be undertaken to follow-up patients until the end of the trial. The percentage of LTFU patients will be determined on Day 28 (primary outcome) and on Day 180.

5 Trial Population

The target patient population consist of adult patients in the ICU or intermediate care unit with sepsis and new, recent onset AKI. Consecutive adult patients with sepsis requiring vasopressor therapy will be systematically screened for AKI as soon as possible following start of vasopressor treatment (see Section 8.2.2). In order to enroll a typical, random sample, reflecting the entry criteria, informed consent will be sought in all patients with AKI and no exclusion criteria.

Only patients with a signed and dated informed consent form (ICF) in compliance with local regulations will be enrolled and randomly assigned to trial drug providing all inclusion criteria and none of the exclusion criteria are met. Deviations from the inclusion and exclusion criteria could potentially jeopardize the scientific integrity of the trial, regulatory acceptability, and, most importantly, patient safety. Therefore, strict adherence to the eligibility criteria as specified in the

protocol is essential. Prospective approval of protocol deviations to recruitment and enrollment criteria, also known as protocol waivers or exemptions, are not permitted.

5.1 Inclusion Criteria

To be eligible for this trial, a patient must meet all of the following inclusion criteria:

1. 18 years or older.
2. In the ICU or intermediate care unit for clinical reasons.
3. Have sepsis requiring vasopressor (norepinephrine, epinephrine, dopamine, phenylephrine, vasopressin, or angiotensin II) therapy, i.e.:
   a) suspected or proven bacterial or viral infection.

   and

   b) on vasopressor therapy (≥0.1 µg/kg/min norepinephrine or equivalent) for sepsis-induced hypotension for at least one hour despite adequate fluid resuscitation according to clinical judgement. Following the initial one hour on at least 0.1 µg/kg/min norepinephrine or equivalent, any dose of vasopressor counts as vasopressor therapy.

   The combination of a) and b) automatically ensures that patients fulfill the Sepsis-3 criteria as 0.1 µg/kg/min norepinephrine corresponds to a score of +4 on the Cardiovascular sub-score of the SOFA score.

4. Have AKI according to at least one of the below KDIGO criteria, a to d:
   a) An absolute increase in serum or plasma creatinine (CR) by ≥0.3 mg/dL (≥26.5 µmol/L) within 48 hours.

      or

   b) A relative increase in CR to ≥1.5 times the pre-AKI reference CR value (see Section 8.3.3.3), which is known or presumed to have occurred within prior 7 days.

      or

   c) A decrease in urinary output to <0.5 mL/kg/hour for a minimum of 6 hours following adequate fluid resuscitation.

      or

   d) If the patient does not have a known history of CKD and there is no pre-AKI reference CR value (see Section 8.3.3.3) available from the past 12 months: a CR value greater or equal to the levels presented in Table 4, with the increase in CR presumed to have occurred within prior 7 days.

Table 4: Gender and Race Corrected Cut-off Values for Serum or Plasma CR Based on 1.5 Times Estimated Normal Values for Age Group[2]

Age (years)	Black males mg/dL (µmol/L)	Other males mg/dL (µmol/L)	Black females mg/dL (µmol/L)	Other females mg/dL (µmol/L)
20-24	2.3 (200)	2.0 (173)	1.8 (159)	1.5 (132)
25-29	2.3 (200)	1.8 (159)	1.7 (146)	1.5 (132)
30-39	2.1 (186)	1.8 (159)	1.7 (146)	1.4 (120)
40-54	2.0 (173)	1.7 (146)	1.5 (132)	1.4 (120)
55-65	2.0 (173)	1.7 (146)	1.5 (132)	1.2 (107)
>65	1.8 (159)	1.5 (132)	1.4 (120)	1.2 (107)

AKI may be diagnosed using any of the above criteria; however, 4d) may only be used if the patient does not have a known history of CKD and there is no pre-AKI reference CR value available from the past 12 months. The purpose of 4d is to allow enrolment of patients when no other pre-AKI CR values are available from which an increase can be documented. Allowing patients to qualify based on a single CR value prevents a delay in enrolment and thereby enables starting treatment as early as possible before irreversible damage occurs. NOTE: an abnormal CR value during hospitalization does not count as pre-AKI reference CR value. Start of AKI is defined as the timepoint where the patient for the first time meets any one of the inclusion criteria 4a)-4d).

5. Provision of signed and dated ICF in accordance with local regulations.

5.2 Exclusion Criteria

A patient who meets any of the following criteria is excluded from participation in this trial:

1.

a) At sites where enrolment of 'moderate' CKD patients is allowed, patients with 'severe' CKD defined as a pre-AKI reference eGFR <25 mL/min/1.73 m^2 are excluded.
   - For patients with known CKD, the most recent eGFR prior to index hospitalization needs to be documented as ≥25 mL/min/1.73 m^2.
   - For patients with known CKD but no known eGFR prior to hospitalization, presentation eGFR between 25-60 mL/min/1.73 m^2 can also be used to rule out 'severe' CKD.

b) At sites where enrolment of 'moderate' CKD patients is NOT allowed, patients with 'moderate' and 'severe' CKD defined as a pre-AKI reference eGFR <45 mL/min/1.73 m^2 are excluded.
   - For patients with known CKD, the most recent eGFR prior to index hospitalization needs to be documented as ≥45 mL/min/1.73 m^2.
   - For patients with known CKD but no known eGFR prior to hospitalization, presentation eGFR between 45-60 mL/min/1.73 m^2 can also be used to rule out 'moderate' and 'severe' CKD.

Due to limited renal reserve, even mild renal insults may trigger the diagnosis of AKI in patients with severe CKD. These "acute on chronic" incidences are often transient with a good prognosis and lower mortality. Therefore, patients with 'moderate' and 'severe' CKD are excluded from the main trial population. However, as CKD patients are more prone to SA-AKI, a limited number of 'moderate' CKD patients with eGFR ≥25-45 mL/min/1.73 m^2 will be enrolled in order to assess the effect of recAP in these patients. NOTE: a recent eGFR value below the thresholds (i.e., below <45 or <25 ml/min/1.73 m^2, respectively) at time of screening does not exclude the patient if the patient does not have a pre-AKI reference eGFR below the required threshold.

2. Advanced chronic liver disease, defined as a Child-Pugh score of 10 to 15 (Class C).
   Patients with advanced chronic liver disease have a very high mortality rate due to their underlying disease, which is unlikely to be influenced by treatment with trial drug.

3. Acute pancreatitis without proven infection.
   Acute pancreatitis may mimic sepsis. Therefore, a proven infection is needed in these patients. Without an established infection, these patients are excluded.

4. Urosepsis related to suspected or proven urinary tract obstruction.
   AKI in urosepsis patients due to obstruction often resolves quickly with no sequela following elimination of the obstruction. As the mechanism of AKI may be different than in classical SA-AKI, these patients are excluded.

5. Main cause of AKI not sepsis.
   If AKI is believed to be due to other causes than sepsis, e.g, nephrotoxic drugs, renal perfusion-related (e.g., acute abdominal aortic aneurysm, dissection, renal artery stenosis) or rhabdomyolysis the patient is excluded as these other causes of AKI have a different pathophysiology that is less likely to be influenced by treatment with recAP.

6. Proven or suspected SARS-CoV-2 infection. NOTE: This exclusion criterion does not apply to patients in the COVID-19 population, in which COVID-19 should be the main cause of SA-AKI.
   At the time of completion of this protocol, there is limited knowledge about the pathophysiology of AKI in COVID-19 patients and associated outcomes. Also, there is currently no experimental data available showing an effect of recAP in these patients. Therefore, patients with proven or suspected SARS-CoV-2 infection are excluded from the main trial population. However, a small separate cohort of COVID-19 patients will be enrolled to provide exploratory data of the effect of recAP in COVID-19 patients.

7. Severe burns requiring ICU treatment.
   Severe burns may resemble sepsis but have special characteristics such as the lack of barrier function, leading to prolonged infection risk, excessive fluid loss, and prolonged

recovery. Therefore, patients with severe burns requiring ICU treatment are excluded from the trial.

8. Severely immunosuppressed, e.g. due to:
    - hematopoietic cell transplantation within past 6 months prior to Screening or acute or chronic graft-versus-host disease
    - solid organ transplantation
    - leukopenia not related to sepsis, i.e., preceding sepsis
    - Human Immunodeficiency Virus (HIV)/Acquired Immune Deficiency Syndrome (AIDS)
    - receiving chemotherapy within 30 days prior to Screening.

    Patients who are severely immunosuppressed have a significantly worse prognosis and a very high mortality rate that may not be related to AKI. Therefore, such patients are excluded from the trial.

9. At high risk of being LTFU, e.g., due to known current or recent (within the last 6 months) IV drug abuse or known to be homeless.

    It is very important to obtain data to the end of the 180-day follow-up period in accordance with the protocol. Therefore, patients at high risk of not showing up to scheduled trial visits and being LTFU are excluded from the trial.

10. Limitations to use of mechanical ventilation (MV), RRT or vasopressors and inotropes (NOTE: limitation of cardiopulmonary resuscitation (CPR) only is not an exclusion criterion).

    Patients who do not wish to receive standard of intensive care with MV, RRT or vasopressors/inotropes are excluded from the trial as these patients are likely to die due to refusal of required organ support. Patients who at time of informed consent allow for active care except CPR can be included in the trial.

11. Previous administration of recAP.

12. Use of a non-marketed drug within the last month or concurrent or planned participation in a clinical trial for a non-marketed drug or device. (NOTE: Co-enrollment or concurrent participation in observational, non-interventional trials using no protocolized treatments or procedures are always allowed. Co-enrollment or concurrent participation in trials using protocolized treatments or procedures, e.g. blood draws, requires pre-approval by the TSC).

    To assess efficacy and safety of recAP without confounding factors, e.g. use of other investigational drugs or devices, co-enrolment in trials involving non-marketed products is prohibited. A non-marketed product is defined as a drug or device that currently do not hold a marketing authorization in any indication. Participation or co-enrolment in purely observational, non-interventional trials using no protocolized procedures are always

allowed. Participation or co-enrolment in trials using protocolized treatments or procedures, e.g. blood draws, requires pre-approval by the TSC and will only be allowed if judged by the TSC to not have an impact on the assessment of efficacy or safety of recAP.

13. Current or planned extracorporeal membrane oxygenation (ECMO).

    These patients also have a very high mortality rate that may not be related to AKI. In addition, they are often transferred to special centers interfering with trial procedures and follow-up in accordance with the protocol.

14. On RRT >24 hours before start of trial drug.

    Only new onset AKI is accepted (see above). Anticipated RRT need following enrolment is NOT an exclusion criterion.

15. No longer on vasopressor therapy at time of randomization.

    The requirement for ongoing vasopressor need despite adequate fluid resuscitation is included to ensure a certain severity of the condition and to align with the patient population of STOP-AKI in which 90% of patients received vasopressor therapy.

16. On continuous vasopressor therapy for >72 hours before start of trial drug.

    Prolonged vasopressor therapy is associated with a risk of organ damage and a poor prognosis. Also, in patients on vasopressors for >72 hours, the link between sepsis and AKI, i.e., SA-AKI is weaker. Therefore, patients having received vasopressor therapy for >72 hours are not eligible. Start of vasopressor therapy is defined as the start time of any dose of vasopressor in the first vasopressor treatment period that includes a continuous infusion of ≥0.1 µg/kg/min norepinephrine (or equivalent) for at least 1 hour for sepsis-induced hypotension in patients who have received adequate fluid resuscitation in accordance with clinical judgement and the recommendations of the Surviving Sepsis Campaign guidelines. A minimum of 12h without any vasopressor is needed to consider start of vasopressor therapy as a new episode. Short-lived vasopressor needs, e.g., during procedures/sedation, does not constitute vasopressor-dependent sepsis.

17. Estimated glomerular filtration rate (eGFR) >60 mL/min/1.73 m^2 based on the most recent available CR sample at time of screening (NOTE: will often be the sample used to diagnose AKI). eGFR should be calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula. In Japan, the CKD-EPI formula with Japanese coefficient should be used.[3] If local regulations prohibit correcting for race in the calculation of eGFR, it is acceptable to use the formula without correcting for race.

    The mortality rate in patients with an eGFR >60 mL/min/1.73 m^2 at time of screening is expected to be low and hence these patients are not suitable for a trial with mortality as the primary endpoint.

18. Not feasible to start trial drug within:
    a) 48 hours from AKI diagnosis, when AKI diagnosis precedes start of vasopressor therapy.

       or

    b) 24 hours from AKI diagnosis, when AKI is diagnosed after start of vasopressor therapy.

    The intention is to start treatment with trial drug as early as feasible to avoid that irreversible organ damage following prolonged AKI prevents the ability to document an effect of recAP. Time of AKI diagnosis is the timepoint of the serum or plasma CR sample or the end of the urine collection period used to establish the AKI diagnosis. Due to the associated risk of ischemia, the risk of permanent organ damage is higher in patients with sepsis-induced hypotension requiring vasopressor therapy, therefore the time window for enrolment is shorter in patients already on vasopressor therapy at time of AKI diagnosis. Patients with AKI without the need for vasopressor therapy are less severely ill and may not yet be in the ICU or intermediate care unit, therefore, the time window for enrolment of these patients is extended to 48 hours from start of AKI.

19. Pregnant or nursing women.

    Reproductive toxicology studies to exclude an effect of trial drug on fetal and postnatal development have not been conducted.

5.3 Lifestyle Considerations

There are no meal, dietary or activity restrictions and no restrictions on caffeine, alcohol and tobacco use.

6 Trial Drug

Trial drug is defined as the investigational drug or matching placebo prepared and supplied specifically for this trial.

6.1 Trial Drugs Administered

Specifications for trial drug administered are summarized in Table 5.

Table 5: Trial Drugs Administered

Trial Drug Name	recAP	Placebo
Dosage Formulation	Clear, colorless to slightly brown, pyrogen-free concentrate for infusion.	Clear, colorless to slightly brown, pyrogen-free concentrate for infusion.
Unit Dose Strength(s)/Dosage Level(s)	An activity of approximately 5,000 U/mL (units of AP activity) at 8.0 mg/mL (protein concentration) in an aqueous buffer at a pH of 7.0 containing: • 3.9 mM histidine • 20 mM citrate • 250 mM sorbitol • 2 mM $MgCl_2$ • 50 µM $ZnCl_2$	An aqueous buffer at a pH of 7.0 containing: • 3.9 mM histidine • 20 mM citrate • 250 mM sorbitol • 2 mM $MgCl_2$ • 50 µM $ZnCl_2$
Route of Administration	Intravenous infusion	Intravenous infusion
Packaging and Labeling	Provided in one pack per patient containing 3 x 6 vials. The vials are 8 mL type 1 glass vials with a Teflon-coated bromobutyl rubber stopper and "Flip-Tear Up" overcap. Vial contains 4 mL of extractable recAP solution. Each glass vial will be labeled as required per country requirement.	Provided in one pack per patient containing 3 x 6 vials. The vials are 8 mL type 1 glass vials with a Teflon-coated bromobutyl rubber stopper and "Flip-Tear Up" overcap. Vial contains 4 mL of extractable placebo solution. Each glass vial will be labeled as required per country requirement.
Manufacturer	Pfizer Inc Puurs Belgium	Pfizer Inc Puurs Belgium

6.2 Preparation/Handling/Storage/Accountability

6.2.1 Trial Drug Preparation

recAP and matching placebo will be provided in one treatment pack per patient containing three sets of vials, one set for each day of treatment. Each set of vials consists of six 8 mL vials with an extractable volume of 4 mL

Prior to administration, the trial drug will be diluted with sterile sodium chloride 0.9% for injection (isotonic saline), USP/EP or equivalent, to a final volume of 50 mL and administered as an IV infusion using an infusion line with an in line filter and dosing syringe or infusion bag. The

intended recAP dose is 1.6 mg (1,000 U) per kg of patient body weight. Patients with a body weight >120 kg will be administered a fixed dose of 192 mg.

Reconstituted trial drug can be stored at 2 to 8°C for up to 24 hours and for a maximum of 8 hours at room temperature and protected from light. If applicable, the trial drug will be re-labeled (as per current regulations) by the hospital pharmacy. Additional details will be provided in the Pharmacy Manual.

6.2.2 Trial Drug Administration

Only patients enrolled and randomized in the trial may receive trial drug. Trial drug will be administered by a 1-hour continuous IV infusion as soon as feasible after randomization on Day 1 and 24 ± 2 hours after the start of the previous drug administration on Day 2 and Day 3, by qualified staff in the ICU or intermediate care unit. Start and stop time and date of each trial drug administration will be collected in the eCRF.

A total volume of 50 mL will be infused at a constant rate of 50 mL/hour, followed by a flushing of infusion lines with a minimum of 4 mL saline. The preferred route for trial drug administration is through a central catheter; if not possible, a peripheral line will be acceptable. Trial drug will be administered separately from any other concomitant drugs using a dedicated lumen of the catheter.

Treatment with recAP will not be repeated in the same patient beyond the scope of this protocol. All patients must be monitored closely for signs of adverse reactions. If administered through a peripheral line, local irritation and hematoma at the infusion site may occur. No specific measures are required if extravasation occurs.

6.2.3 Trial Drug Handling and Storage

Receipt and handling of trial drug will be in accordance with local Standard Operating Procedure (SOP). Storage will be in a lockable, monitored storage facility with access limited to authorized staff only. Trial drug vials are to be stored at 2°C to 8°C and protected from light and moisture until preparation for use.

Full details of trial drug packaging, storage, and shipment will be provided in the Pharmacy Manual. Trial drug will be packaged and labeled in accordance with Good Manufacturing Practice (GMP), Good Clinical Practice (GCP) and local requirements.

6.2.4 Trial Drug Accountability

The Investigator or designee has the responsibility for the following:

- Maintaining accurate records of receipt of all trial drug, including dates of receipt at the ICU or intermediate care unit.
- Keeping accurate records regarding when and how much trial drug is dispensed and administered to each patient in the trial. The drug supplied for this trial may only be administered to patients in this trial.
- Recording reasons for deviating from the expected dispensing regimen as instructed.

6.3 Measures to Minimize Bias: Randomization and Blinding

6.3.1 Randomization

Eligible patients are assigned a unique patient identification number via Interactive Response Technology (IRT) randomly allocating each patient to active or placebo according to the randomization schedule generated by a validated computer program. Details of the procedure are described in the IRT Manual provided to all sites.

Patients are randomly assigned 1:1 to receive either recAP or placebo.

Randomization will be stratified by:

- 'Moderate' CKD defined as a pre-AKI reference eGFR ≥25 and <45 mL/min/1.73 m^2
  - Yes
  - No
- Baseline mSOFA score, i.e., excluding the GCS part.
  - mSOFA score ≤9
  - mSOFA score >9
- Clinical site

Randomization for the COVID-19 population will be stratified by:

- Baseline mSOFA score, i.e., excluding the GCS part.
  - mSOFA score ≤9
  - mSOFA score >9
- Clinical site

6.3.2 Blinding

In general, all persons involved in the trial (including but not limited to the patient, site staff, the Sponsor or its designee team members) will be blinded to trial drug assignment. The randomization schedule for the main trial population will be kept by the IRT provider and will not be revealed until all patients in this population have completed Day 90 and the interim lock for all patients in this population has been performed, after which it will be released to the trial statistician, while all other persons involved in the trial remain blinded to the individual patient treatment allocation. Likewise, the randomization schedule for all patients in the 'moderate' CKD population will be kept by the IRT provider and will not be revealed until all patients in this population have completed Day 90 and the interim lock for these patients has been performed after which it will be released to the trial statistician, while all other persons involved in the trial remain blinded to the individual patient treatment allocation. Unblinding of patients in the 'moderate' CKD population will only take place after unblinding of patients in the main trial population if separate database locks (DBLs) for these two patient populations are performed.

The unblinded outputs for the safety data review and the interim analysis reports for the DMC will be prepared by an unblinded statistician not otherwise involved in the trial. The unblinded statistician will also perform the analysis of the primary endpoint following the Day 28 database snapshot and report the result to the Sponsor in a blinded manner. Furthermore, the unblinded

statistician will be responsible for analyzing unblinded data from the COVID-19 population if this cohort of patients complete the trial at any time prior to the final DBL. In the event of a Quality Assurance Audit, the auditor(s) will be allowed access to blinded trial drug records at the site(s) to verify that randomization/dispensing has been done accurately.

To avoid unblinding of the trial team, formal approved site-specific blinding plans need to be in place before the initiation visit to prevent measuring and reporting of AP activity plasma levels as part of the routine clinical chemistry panel. This measure must be in place up to and including Day 14, irrespective if the patient is in the ICU, in the intermediate care unit or in the general ward. Measuring and reporting AP activity could not only lead to unblinding of staff but might also lead to erroneous interpretation of liver function, as administration of recAP will lead to increased AP activity levels, to values significantly exceeding the upper limit of the reference normal range. For that reason, AP activity levels should not be measured from samples obtained during the first 14 days of the trial. If measured as part of a standard package, the results should not be reported. Any reporting of AP activity levels is considered an important protocol deviation. After Day 14, AP activity levels are expected to have returned to normal considering the elimination half-life of recAP (~59 hours in patients with SA-AKI). Results of AP activity in samples taken after Day 14 may be reported.

Antibody data and PK data (other than date and time of collection) will be maintained by personnel unblinded to the trial for this purpose, but not involved in the trial otherwise.

Full details of the handling of restricted and potentially unblinding data will be documented.

6.3.2.1 Breaking the Blind

A patient's treatment assignment will not be broken until the database is formally unblinded unless knowledge of the trial medication that the patient received is required to guide medical treatment of the patient. In case of an emergency, the Investigator has the sole responsibility to decide if unblinding a patient's trial drug assignment is warranted and to unblind an individual patient's treatment allocation. The Investigator must contact the Medical Monitor to discuss the medical emergency and the reason for revealing the actual treatment unless immediate unblinding is necessary to ensure the patient's safety.

The treatment assignment will be unblinded through the IRT system. Reasons for treatment unblinding must be recorded and justified in the eCRF and IRT. In case a patient's trial drug assignment is unblinded, the Sponsor or its designee must be notified within 24 hours after the blind is broken and trial drug administration to the patient should be permanently stopped. The date on which the blind was broken and the identity of the person responsible must be documented.

The IRT system will be programmed with instructions for breaking the blind.

6.4 Trial Drug Compliance

Trial drug will be administered in the ICU/and or intermediate care unit by the Investigator or qualified personnel and the dates and times of administration will be recorded in the eCRF and drug accountability trackers.

In case the patient has been discharged from the ICU or intermediate care unit to a ward within the hospital before completing the last trial drug administration on Day 3, trial drug should be administered at the ward by qualified personnel following instructions provided by the trial team.

Drug accountability details are provided in Section 6.2.4. Instructions on how to act in case of an overdose are provided in Section 8.4.

6.5 Prior and Concomitant Medications

Concomitant medication may be administered, and procedures may be conducted for the well-being of the patient at the discretion of the Investigator. Use of vasopressors, inotropic drugs, MV and RRT must be documented in detail as these are related to the endpoints of the trial. In addition, nephrotoxic drugs and prior and concomitant medications and procedures that might influence the outcome of SA-AKI must be detailed.

With the exception of first IV antibiotic(s) for sepsis, prior medication(s) should only be collected from time of Baseline. Concomitant medication should be collected from start of trial drug administration on Day 1 to Day 28 or until hospital discharge, if before Day 28.

The following prior and concomitant medication must be collected:

- Corticosteroids:
  - for septic shock: generic name, start and stop date and maximum dose
- IV antibiotics:
  - first IV antibiotic: generic name, start date and time, stop date, indication incl. suspected or confirmed microbial agent
  - subsequent IV antibiotic: generic name, start date and stop date, indication incl. suspected or confirmed microbial agent
- Nephrotoxic drugs: generic name, start and stop date and maximum dose
- Vasopressor and inotropic drugs: see recording details in Section 8.3.3.7
- MV: see details in Section 8.3.3.5
- RRT: see details in Section 8.3.3.6

In case of an AE or SAE all concomitant medication of relevance to the event must be collected.

All patients in this trial will receive supportive care according to best practice. The management of a patient should be based on the Surviving Sepsis Campaign guideline for sepsis and KDIGO Clinical Practice Guidelines for AKI[5, 44].

The Medical Monitor must be contacted if there are any questions regarding concomitant or prior medications.

6.5.1 Concomitant Medications to be Avoided

After randomization, the administration of nephrotoxic drugs, such as contrast agents, non-steroidal anti-inflammatory drug (NSAIDs), angiotensin-converting enzyme inhibitors, gentamycin, or tobramycin should be avoided where possible, as recommended by the KDIGO Clinical Practice Guideline for AKI recommendations[44].

6.6 Dose Modification

Not applicable for this trial.

6.7 Intervention After the End of the Trial

Not applicable for this trial.

7 Discontinuation of Trial Drug and Patient Discontinuation

7.1 Discontinuation of Trial Drug

Administration of trial drug should be stopped for any of the following reasons:

1. If in the opinion of the Investigator, an SAE indicates that continued treatment with trial drug is not in the best interest of the patient. In such cases, monitoring of the patient will continue until the event has resolved or stabilized or until a determination of a cause unrelated to the trial drug or trial procedure is made. The specific event or laboratory finding(s) must be documented in the eCRF.
2. The patient withdraws consent to receive trial drug or withdraws consent to trial participation, or the Investigator or Sponsor decide to discontinue the patient's participation in the trial.
3. After emergency unblinding of a patient.
4. The Sponsor terminates the trial.
5. Pregnancy (see Section 8.3.1.4).

In case trial drug administration is discontinued prematurely, the patient should still continue all planned follow-up assessments as per protocol for safety and to allow for analysis of the specified endpoints. If trial drug administration is interrupted for any reason, re-starting of trial drug must be discussed with the Medical Monitor.

The reason for and date of trial drug discontinuation must be recorded in the eCRF.

7.2 Patient Withdrawal from the Trial

Patients may withdraw from the trial at any time and for any reason without explanation or prejudice to their future medical care by the Investigator or at the trial site. Moreover, a patient may be withdrawn from the trial at any time at the discretion of the Investigator for safety, behavioral or administrative reasons.

The extent of a patient's withdrawal from the trial (e.g., withdrawal from receiving further trial drug, trial procedures, data collection, withdrawal from further contact) and the cause of withdrawal must be documented in the eCRF. Efforts must be made to follow-up withdrawn patients to the extent that the patient agrees to, i.e., if a patient only withdraws from receiving further trial drug, procedures and data collection should still be performed in accordance with the protocol. All patients who withdraw consent to future procedures should, if at all possible, have the EOT assessments performed immediately upon withdrawal. Unless the patient specifically withdraws from all further contact, the patient or his/her legal representative will be contacted by telephone on Day 28, Day 90 and Day 180 to verify if the patient is alive. If local regulations permit, and consent has been obtained, registries may be used for obtaining survival data for patients who withdraw from all further contact.

It is highly desirable to obtain follow-up safety data on patients withdrawn because of an SAE. In every case, reasonable efforts must be made to undertake protocol-specified, safety follow-up procedures (Section 8.3.4.4) to the extent that the patient agrees to.

The Sponsor may retain and continue to use any data collected prior to the withdrawal of consent. If a patient withdraws from the trial, he/she may request destruction of samples taken and not yet tested, and the Investigator must document this in the site trial records.

Refer to the SoA (Table 3) for data to be preferably collected at the time of withdrawal from the trial.

7.3 Loss of Patients to Follow-Up

A patient will be considered LTFU if he or she repeatedly fails to return for scheduled visits and the trial site is not able to establish contact.

The following actions must be taken if a patient does not show up for a trial visit:

- The site must attempt to contact the patient and reschedule the missed visit as soon as possible (and within the visit window) and counsel the patient on the importance of adhering to the assigned visit schedule.
- When a patient is suspected LTFU, the Investigator or designee must make the following efforts to regain contact with the patient (a minimum of three telephone calls and, if necessary, a certified letter to the patient's last known mailing address or local equivalent methods or a home visit by a third vendor, if allowed by local regulations). These attempts are considered a reasonable effort to obtain information before a patient is considered definitely LTFU. The attempts need to be documented in the patient's medical records.
- When a patient remains unreachable, he/she will be considered LTFU from the trial.

8 Screening and Enrollment, Trial Days, Trial Assessments and Procedures

8.1 Pre-screening, Screening and Enrolment

To efficiently gather a random sample reflecting the entry criteria and thus the target population, consecutive screening and enrolment is used. By systematically considering all relevant patients, arbitrary selection, i.e., exclusion of patients unrelated to the eligibility criteria, is prevented. This is essential to be able to apply the trial outcome to clinical practice as evidence-based medicine. The principle of consecutive screening and enrolment has proven valuable in other clinical trials[45].

All adult patients in the ICU or intermediate care unit with sepsis requiring ≥0.1 µg/kg/min norepinephrine (or equivalent) for at least one hour should be systematically evaluated for AKI (see the "Pre-screening, screening and enrolment guide" for a detailed description of the systematic approach that should be applied to identify and assess patient eligibility for the trial).

The systematic and consecutive procedure ensures that all patients that could be relevant are considered and that all potentially medically eligible patients with AKI within the relevant time window will be identified. The procedure also ensures that all patients meeting any exclusion criteria can be accounted for.

Informed consent will be pursued in all patients fulfilling the medical inclusion criteria and not meeting any exclusion criteria. Informed consent must be obtained in accordance with local regulation before any trial-specific procedures are to be performed (see Section 10.1.3 for further details on the consent process). Each patient with a signed and dated (incl. time) ICF according to local regulations will be assigned a unique patient identification number (see Section 6.3.1 for further details).

After signed informed consent has been obtained in accordance with local legislation, trial specific assessments will be completed and reviewed to confirm that the patient meets all eligibility criteria. In case a patient does not meet the eligibility criteria, he/she will be considered a screen failure. The reason for screen failure will be recorded in the eCRF. Thus, a screen failure defines a patient with consent to participate but who will not subsequently be randomly allocated to trial drug.

Minimal information on screen failures is required to ensure transparent reporting and meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, eligibility criteria/reason for exclusion, any SAEs and date of ICU or intermediate care unit admission.

A 24/7 medical hotline will be available to assist sites with medical questions related to eligibility and other trial related medical questions. For the first two patients enrolled at each site, a call to the 24/7 medical hotline to discuss eligibility is mandatory.

8.2 Trial Days

8.2.1 Pre-screening, Screening and Baseline

Pre-screening is defined as the process whereby the screen population with sepsis requiring vasopressor therapy is systematically evaluated for having AKI, without performing any trial specific procedures requiring informed consent. Pre-screening takes place during the screening period, which lasts from the timepoint when the patient meets the criteria for belonging to the screen population and until the time window for potential eligibility ends. Thus, patients may be pre-screened multiple times and a patient who is not eligible at the initial pre-screening may become so later. For details, see the trial specific “Pre-screening, screening and enrollment guide”. Obtainment of informed consent defines the transition from pre-screening to screening.

Baseline is the time period from meeting all eligibility criteria to start of trial drug. During this period, all Baseline assessments will be performed, and the patient will be randomly allocated to a treatment group.

The assessments to be performed at Baseline are listed in the Section 8.3 and in the SoA (Table 3).

8.2.2 Day 1 to Day 3: Treatment Days

Trial drug administration will start as soon as possible after randomization on Day 1 and 24 ± 2 hours after the start of the previous drug administration on Day 2 and Day 3 (further details are provided in Section 6.2.2).

When AKI diagnosis precedes start of vasopressor therapy, start of trial drug must be within 48 hours from AKI diagnosis. When AKI diagnosis is made after start of vasopressor therapy, trial drug must be started within 24 hours from AKI diagnosis and no longer than 72 hours after start of vasopressor therapy (see Section 6.2.2 for further details).

The assessments performed on Days 1 to Day 3 are listed in Section 8.3 and in the SoA (Table 3).

8.2.3 Days 4, 5, 6, 7, 28, 90, and 180 Assessments and Procedures

Assessments and procedures to be performed on Days 4, 5, 6, 7, 28, 90 and 180 are listed in Section 8.3 and in the SoA (Table 3).

The Day 28 visit is scheduled 27 days after first trial drug administration, with a time window of maximum +4 days (i.e., on Day 28, Day 29, Day 30, Day 31 or Day 32). The Day 90 and Day 180 visits are scheduled 89 and 179 days, respectively, after first trial drug administration with a time window of maximum +10 days. Day 180 is considered the end of the trial.

The assessments may be performed in the hospital for patients who are in the hospital or who are able to come to the hospital after discharge or at home if the patient is not able to visit the hospital after discharge. Home visits can be made by trial personnel or by a third vendor, if allowed by local regulations. The Day 180 visit can be conducted by phone unless a blood sample for ADA is needed.

8.3 Trial Assessments and Procedures

- Trial procedures and their timings are summarized in the SoA, Table 3.
- Protocol waivers will not be granted.
- If site personnel deviate from the protocol, Labcorp (the Sponsor's CRO, previously known as Covance) must be notified immediately on the occurrence or on becoming aware of it to provide guidance how to proceed.
- The maximum amount of blood collected for trial specific assessments from each patient over the duration of the trial will not exceed 100 mL when excluding blood collection for assessments considered standard clinical practice. The total amount of blood collected from each patient over the duration of the trial, i.e., 180 days including samples taken as part of standard clinical practice is not expected to exceed 300 mL.
- Repeat or unscheduled samples may be required for safety reasons or for technical issues with the samples.
- Procedures conducted as part of the patient's routine clinical management (e.g., laboratory results, height and weight) which are obtained before signing of ICF may be used for screening or baseline purposes.

8.3.1 Eligibility/Screening Assessments

Patients with a diagnosis of SA-AKI who fulfill all eligibility criteria (see Section 5) are eligible for the trial. For a detailed description of the screening process, see the trial specific "Pre-screening, screening and enrollment guide".

8.3.1.1 Diagnosis of Sepsis Requiring Vasopressor Therapy

Sepsis will be diagnosed according to the Sepsis-3 criteria. Patients with suspected or proven bacterial or viral infection who receive vasopressor therapy (≥0.1 µg/kg/min norepinephrine or equivalent) comply with the Sepsis-3 criteria as 0.1 µg/kg/min norepinephrine corresponds to a score of +4 on the Cardiovascular sub-score of the SOFA score. The date and time of fulfilling inclusion criteria 3 should be documented in the eCRF.

8.3.1.2 Diagnosis of Acute Kidney Injury

AKI may be diagnosed by any one of the criteria 4a) to 4d) listed in Section 5.1 using serum or plasma CR values or urine output values obtained for clinical purposes. However, criterion 4d) may only be used in patients with no known history of CKD and/or no pre-AKI reference CR value available within the past 12 months. The timepoint of first meeting any of the four criteria defines the time of AKI diagnosis. If the AKI diagnosis is made by measurement of serum or plasma CR, it is the time when the diagnostic blood sample was drawn that counts as the time of AKI diagnosis. If the AKI diagnosis is made based on urine output, it is the end of the collection period that counts as the time of AKI diagnosis.

Assessment of AKI status should take place as soon as possible after the patient has received ≥0.1 µg/kg/min norepinephrine (or equivalent) for at least one hour for sepsis-induced hypotension to enable early enrolment and start of trial drug administration if the patient is medically eligible and informed consent is obtained.

If a patient qualifies on more than one of the criteria 4a) to 4d), all should be recorded. The CR and/or urine output values used for establishing the AKI diagnosis and the date and time of sampling should be documented in the eCRF. Time of AKI diagnosis will be derived from the respective information.

8.3.1.3 Informed Consent

Informed consent is to be signed by the patient, his/her legal representative, an independent consulting physician, or obtained based on an emergency trial protocol by the Investigator where country regulations and institution guidelines allow this. The informed consent procedure must be approved by the Institutional Review Board (IRB)/ Independent Ethics Committee (IEC) and/or national authorities. Incapacitated patients enrolled in the trial must sign the most recent ICF as soon as they are capable to do so.

8.3.1.4 Pregnancy Test

A serum or urine human chorionic gonadotropin (hCG) pregnancy test will be performed for all women of childbearing potential (WOCBP). The pregnancy test will be performed at the local hospital laboratory or at the ICU/intermediate care unit.

A woman is considered of childbearing potential when she is fertile, i.e., following menarche and until becoming post-menopausal unless permanently sterile. Permanent sterilization methods include hysterectomy, bilateral salpingectomy, and bilateral oophorectomy. A postmenopausal state is defined as no menses for 12 consecutive months without an alternative medical cause. If menopausal state is unknown, women ≤55 years will be considered of childbearing potential.

8.3.2 Demographics and Other Baseline Assessments

8.3.2.1 Demographics

Demographic information including gender, age, ethnicity and race, will be recorded. If local regulations prohibit collection of information on ethnicity and/or race, missing data for these parameters will not be considered protocol deviations.

8.3.2.2 Medical History

Medical history will be recorded by the Charlson co-morbidity index. The Charlson comorbidity index predicts the one-year mortality for a patient who may have a range of comorbid conditions, such as heart disease, AIDS, or cancer (a total of 19 conditions). Each condition is assigned a score of 1, 2, 3, or 6, depending on the risk of dying associated with each condition. Scores are summed to provide a total score to predict mortality. In addition, recent medical history within the past 30 days considered relevant to the current episode of SA-AKI must be collected including whether the reason for ICU or intermediate care unit admission is medical, surgical or trauma. Special attention must be given to pre-existing renal disease and use of immunosuppressant medication, steroids and nephrotoxic drugs. The relevant medical history must be fully documented in the patient's hospital records and in the eCRF.

8.3.2.3 Weight and Height

Weight and height will be determined or estimated and used to calculate the body mass index (BMI) of the patient. The hospital admission weight (or if unavailable an estimated weight) in kg will be used for reconstitution of the trial drug (see Section 6.2.1). The same weight will be used on all treatment days.

8.3.2.4 12-lead Electrocardiogram

A 12-lead electrocardiogram (ECG) obtained prior to start of trial drug (Baseline ECG) must be captured in source documents. ECGs obtained for clinical purposes within 48 hours of starting the trial drug may be used as Baseline ECG, provided there has been no clinically significant change in the cardiac status between the time of its recording and the start of the trial drug administration. If there has been a clinically significant change in cardiac status, a new 12-lead ECG must be obtained prior to start of the trial drug administration. If prone positioning ventilation prevents the recording of a 12-lead ECG, a 3-lead ECG will suffice. The purpose of the Baseline ECG is to have a status at entry to which later ECGs may be compared, should this become relevant.

8.3.2.5 Acute Physiology and Chronic Health Evaluation (APACHE) II Score

The Acute Physiology and Chronic Health Evaluation (APACHE) II score is a severity-of-disease classification system, used in ICU or intermediate care unit settings. A score from 0 to 71 is calculated from 12 physiological measurements using the worst value within the past 24 hours. Higher scores indicate more severe disease and higher mortality risk. The score should be calculated based on values from the 24 hours up to time of randomization.

- To calculate the total APACHE II score, parameters for vital signs, oxygenation, clinical chemistry and hematology are required. Locally measured clinical chemistry and hematology results may be used.
- If more than one result for a certain parameter is available, the worst result must be used.
- The GCS should be obtained preferably before sedation of the patient. If the patient is sedated, the last value before sedation should be used.

8.3.2.6 SOFA Score

See Section 8.3.3.4. Patients are stratified according to the mSOFA score (≤9 or >9).

8.3.2.7 KDIGO AKI Stage

See Section 8.3.3.3. The KDIGO AKI severity stage will be assessed at Baseline.

8.3.2.8 Site of Infection and Pathogen Causing Sepsis

The source of sepsis will be determined on the basis of microbial tests performed by the local laboratory. The following information related to the main cause of sepsis must be recorded in the eCRF:

- Whether the infection is proven or suspected at time of randomization.
- Known or suspected infection site and pathogen at time of randomization.
- In case of suspected infection at the time of randomization, final conclusion on infection site and pathogen when the data becomes available.

8.3.2.9 Other Baseline Assessments

For more details on CR to calculate eGFR, hematology and clinical chemistry samples, biomarkers, ADA samples and Functional status and residency, see Section 8.3.3.

8.3.3 Efficacy Assessments

8.3.3.1 Mortality

The date and cause of death up to Day 180 will be recorded on a separate page in the eCRF. In addition, the underlying cause of death must be reported as an SAE, for deaths until Day 28 (incl.) (see Appendix 2).

If a patient is discharged from the ICU and/or hospital or when a patient is not able to return for Day 28, Day 90 and/or Day 180 visits, the Investigator or designee must make reasonable efforts to contact the patient, the legal representative, General Practitioner or institute to which the patient has been transferred, to obtain information on the patient being alive, or by consulting national registries if available. See Section 7.3 for information with regards to patients LTFU. The contact attempts will be documented in the source data.

When information on Day 180 survival status can be obtained, the patient is not considered LTFU.

8.3.3.2 Serum or Plasma Creatinine Levels and eGFR Calculation

Serum or plasma CR levels will be measured locally at the ICU or local hospital laboratory at Baseline, daily up to Day 7 (or until ICU or intermediate care unit discharge if the patient leaves the ICU or intermediate care unit before Day 7), on Day 28 and Day 90. A CR sample taken less than 12 hours before randomization as part of clinical practice is allowed to be used as Baseline (see Section 8.3.4.2) and for eGFR calculation. If multiple values are available, the value closest to the timepoint of randomization should be used. If Day 1 and Baseline is on the same calendar day, there is no requirement to repeat measurements on Day 1 after trial drug administration. However, if CR is measured for clinical purposes on Day 1 after trial drug administration, the result should be entered in the eCRF. From Day 2 onwards, the result of the worst daily sample

should be recorded if multiple values are available. Collection times and dates will be recorded in the eCRF. In the event a patient is offered a home visit on Day 28 and/or Day 90, the home nurse/trial personnel will collect the sample for creatinine which may be returned via courier to the central laboratory for analysis.

The CKD-EPI formula using serum or plasma CR[46, 47] will be used for the eGFR calculation. In Japan, the CKD-EPI formula with Japanese coefficient will be used.[3]

8.3.3.3 KDIGO AKI Stage

The KDIGO AKI severity stage will be assessed at Baseline, daily up to Day 7 (or until ICU or intermediate care unit discharge if the patient leaves the ICU or intermediate care unit before Day 7), on Day 28 and Day 90 using the CR values collected as described in Section 8.3.3.2 or using urine output measurements, if available. If both CR and urine output criteria for AKI diagnosis are met, the most severe AKI stage must be used and recorded in the eCRF. The pre-AKI reference CR value should also be recorded. The pre-AKI reference value refers to the patient's usual CR/eGFR level before developing AKI. The pre-AKI reference value is defined as the median of the 3 most recent CR/eGFR values in the past 12 months before developing AKI. For patients with known CKD, the median of values covering at least 3 months should be used. If less than 3 values are available, the most recent value is to be used. The same pre-AKI reference CR value should be used for all calculations.

Table 6: KDIGO AKI Staging

Stage	Serum creatinine	Urine output
1	1.5 – 1.9 times known or assumed pre-AKI reference value[1] OR ≥0.3 mg/dL (≥26.5 µmol/L) increase	<0.5 mL/kg/h for 6-12 hours
2	2.0 – 2.9 times known or assumed pre-AKI reference value[1]	<0.5 mL/kg/h for ≥12 hours
3	3.0 times known or assumed pre-AKI reference value[1] OR Increase in serum CR to ≥4.0 mg/dL (≥353.6 µmol/L) OR Initiation of renal replacement therapy	<0.3 mL/kg/h for ≥24 hours OR Anuria for ≥12 hours

Modified from: Kidney Disease Improving Global Outcomes (KDIGO) Clinical Practice Guideline for Acute Kidney Injury (2012).

[1]: If the pre-AKI reference CR value is unknown, the following normal values for the age groups may be used as

assumed reference values:

Age (years)	Black males mg/dL (µmol/L)	Other males mg/dL (µmol/L)	Black females mg/dL (µmol/L)	Other females mg/dL (µmol/L)
20-24	1.5 (133)	1.3 (115)	1.2 (106)	1.0 (88)
25-29	1.5 (133)	1.2 (106)	1.1 (97)	1.0 (88)
30-39	1.4 (124)	1.2 (106)	1.1 (97)	0.9 (80)
40-54	1.3 (115)	1.1 (97)	1.0 (88)	0.9 (80)
55-65	1.3 (115)	1.1 (97)	1.0 (88)	0.8 (71)
>65	1.2 (106)	1.0 (88)	0.9 (80)	0.8 (71)

8.3.3.4 Modified Sequential Organ Failure Assessment Score (mSOFA score)

The development of organ failure over time will be assessed by the mSOFA scores daily from Baseline to Day 7 (or until the patient is discharged from the ICU or intermediate care unit if before Day 7). The full SOFA score is composed of six sub-scores; one for each of the respiratory, cardiovascular, hepatic, coagulation, renal and neurological systems[48, 49]. The neurological score (based on GCS) will not be included in the calculation of the mSOFA score, since many patients will be sedated leading to a potentially misleading GCS score.

The mSOFA score calculation requires hematology (i.e., platelets) and clinical chemistry measurements (i.e., bilirubin and CR). On days where hematology and clinical chemistry measurements are required as part of safety measurements, these results can be used for calculation of the mSOFA score. On days where hematology and clinical chemistry measurements are not required, the platelets, bilirubin, and CR must be measured by the local laboratory to calculate a mSOFA score. If Day 1 and Baseline is on the same calendar day, mSOFA does not need to be obtained again on Day 1 after trial drug administration. Results of assessments done for clinical purposes on Day 1 after trial drug administration should be entered in the eCRF.

All underlying parameters of the mSOFA score(s) must be recorded in the eCRF.

8.3.3.5 Mechanical Ventilation

Mechanical ventilation will be recorded from Baseline up to Day 28, included.

Mechanical ventilation is defined as any positive pressure ventilation via endotracheal or tracheostomy tube or any non-invasive ventilation with >5 cm H_2O pressure. In patients ventilated via endotracheal tube, end of MV is defined as extubation. In patients ventilated via tracheostomy, end of MV is defined as removal of the tracheostomy tube or reduction to ≤5 cm H_2O pressure (regardless of modality). Similarly, in non-invasively ventilated patients, end of MV is defined as reduction to ≤5 cm H_2O pressure (regardless of modality).

In case the patient needs re-intubation or re-start of non-invasive ventilation with >5 cm H_2O pressure (regardless of modality) within 48 hours after end of MV, the ventilation period continues and the days (<2 days) in between are considered as MV days.

The use of MV associated with anesthesia/sedation during and up to three hours after surgery or a procedure, will not count as MV. Also, night-time use of continuous positive airway pressure (CPAP) or bilevel positive airway pressure (BiPAP) for chronic obstructive pulmonary disease (COPD) or sleep apnea does not count as MV.

MV start and stop dates and times together with information on the modality/modalities will be recorded in the eCRF.

8.3.3.6 Renal Replacement Therapy

All modalities of RRT are allowed in the trial, including continuous, intermittent and other non-continuous RRT modalities. The time on RRT will be assessed from Baseline up to Day 28 included. In addition, at Day 90, the RRT status (i.e., not on RRT, on RRT for AKI, or permanently dialysis-dependent for end-stage renal disease) will be recorded.

Initiation of RRT should preferably be based on the below criteria as described by Bellomo et al (2012)[50]. A patient who meets at least one of the below criteria will be eligible for RRT initiation:

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 CR concentrations 300 µmol/L).
7. Clinical complications of uremia (e.g., encephalopathy, pericarditis, neuropathy).

While the above criteria for starting RRT are strongly preferred, Investigators may use other criteria based on clinical judgment and standard practice. The main reason for RRT initiation will be recorded in the eCRF, as well as the modality and the start and stop dates of RRT. Modalities other than continuous RRT (CRRT) are defined for purposes of this protocol as intermittent RRT (IRRT).

8.3.3.7 Use of Vasopressors and/or Inotropic Drugs

The use of vasopressors and/or inotropic drugs will be recorded on a daily basis from Baseline to Day 28.

For inotropic and vasopressor agents, the generic name, start and stop date and time and the daily maximum dose will be recorded in the eCRF. The reason for vasopressor/inotropic administration will be recorded by discriminating between, “use of vasopressor/inotropes for sepsis-induced hypotension/myocardial dysfunction” or “use of vasopressors /inotropes during and up to three hours after surgery/procedure”. The “use of vasopressors /inotropes during and up to three hours after surgery/procedure” will not count as vasopressor/inotropic use in the statistical analysis of the associated endpoints.

8.3.3.8 Quality of Life EQ-5D-5L Questionnaire

The Quality of Life (QoL), EQ-5D-5L, is a standardized measure of health. If a patient is unable to complete the questionnaire, a member of the trial team can interview the patient by reading the questions and answers objectively. Each questionnaire provided to a patient must contain the date of the assessment and patient number and be checked for completeness.

The QoL EQ-5D-5L will be performed at Baseline, Day 28, Day 90 and Day 180. The EQ-5D-5L for Baseline refers to a recall of the patient’s situation before SA-AKI occurred and must be completed by the patient him/herself and not by e.g., a family member. If the patient is unable to complete the questionnaire, e.g., due to sedation, it should be completed as soon as the patient is

capable. If the patient is discharged from hospital, the questionnaire may be completed by a phone interview. Responses will be recorded in the eCRF.

8.3.3.9 Functional Status and Residence

Functional status and residence of the patient prior to the hospitalization for SA-AKI as well as functional/survival status and residence on Day 28, Day 90 and Day 180 will be recorded.

The assessment will be performed by ticking one of the possibilities in the eCRF.

- Home, receiving no support
- Home, receiving paid/unpaid support
- Rehabilitation site
- Nursing facility
- Other acute care hospital (including long-term acute care)
- Still in (or readmitted to) trial hospital
- Unknown
- Dead

8.3.3.10 Length of Intensive Care Unit and Hospital Stay and Rehospitalization

The admission and discharge date and the reason for admission to the hospital and ICU or intermediate care unit will be recorded in the eCRF.

The following data for all-cause rehospitalization to any hospital will be collected up to visit Day 90:

- Admission and discharge date.
- The reason(s) for rehospitalization.

Rehospitalization is defined as any overnight stay in a hospital. If the patient is discharged from the hospital/ICU or intermediate care unit, the required information must be obtained from the patient, a family member or the general practitioner.

8.3.3.11 Biomarkers

Blood samples for biomarker determinations will be collected every day from Baseline to Day 5 included, and on Day 28. Plasma samples will be stored up to five years after the final Clinical Trial Report (CTR) is published for the determination of biomarkers including new biomarkers, which may come available in the sepsis and/or AKI field (see the Laboratory Manual for further information).

<u>Purines</u>

The collection of urine samples to determine purine levels will take place in selected sites. Purines (ATP, ADP, AMP, cAMP and adenosine) and CR will be measured from urine samples obtained every day from Baseline up to Day 4 included. At the dosing days (Day 1, Day 2 and Day 3), a post-dose urine sample must be taken within one hour after the end of trial drug administration. On Day 4, urine collection can be performed at any time.

A spot sample should be taken directly from the urine catheter in order to avoid degradation. The urine flow rate will be calculated from the urine volume collected for 1 to 2 hours after the spot sampling. The respective start date/time (date/time of spot sampling) and stop date/time of the urine collection and the volume collected will be recorded in the eCRF.

Processing of the urine samples is described in detail in the Laboratory Manual.

Samples will be stored up to 5 years after the final CTR is published for the determination of urinary biomarkers including new biomarkers, which may come available, in the sepsis and/or AKI field.

8.3.4 Safety Assessments

8.3.4.1 Prior and Concomitant Medication

Details of prior and concomitant medication to be recorded in the eCRF are summarized in Section 6.5.

In case of an AE or SAE, information on all concomitant medication of relevance to the event must be recorded.

8.3.4.2 Clinical Chemistry and Hematology

Blood will be collected by qualified personnel according to standard procedures at the timepoints presented in the SoA, Table 3. See Section 8.3.4.4.4 for additional details on pregnancy.

Blood samples will be collected for local laboratory measurements at Baseline, Day 3 and Day 28 or at hospital discharge if before Day 28, and at EOT if a patient withdraws before Day 28 (see Table 3). Note that platelets, total bilirubin, CR, and urine output are required to calculate the mSOFA score.

The tests detailed in Table 7 will be performed by the local laboratory. The local laboratory testing panel may include additional results; those listed in this table are minimum requirements to be assayed and are expected to be entered into the eCRF.

NOTE: Alkaline phosphatase activity measurement and reporting is **NOT** allowed from the first dosing with the trial drug up to and including Day 14. If for example, automated AP activity measurements cannot be avoided, the results from blood samples taken during the first 14 days of the trial could lead to erroneous interpretation of liver function, as recAP administration will increase the measured AP activity. AP activity results in this period are then not to be reported to trial team members or to any other blinded personnel involved in patient care and/or data collection as this could lead to unblinding (see Section 6.3.2).

Table 7: Protocol-Required Safety Laboratory Assessments

Laboratory Assessments	Parameters	
Hematology	Red blood cell Count Hemoglobin (Hb) Hematocrit (Ht) Platelet count	White blood cell count with Differential (absolute counts): • Neutrophils • Lymphocytes • Monocytes • Eosinophils • Basophils
Clinical Chemistry	Blood urea nitrogen Potassium Chloride Bicarbonate Total bilirubin CR Sodium Total Protein C-reactive protein (CRP) Albumin	Glucose (non-fasting/fasting) Aspartate Aminotransferase (AST) Alanine Aminotransferase (ALT) Lactate dehydrogenase (LDH) Gamma-glutamyl transpeptidase (GGT) Lactate D-dimer (only patients in the COVID-19 population)

The Investigator must review the laboratory reports, document the review, and record any clinically relevant changes considered more severe than expected for the patient's medical condition in the AE section of the eCRF. The laboratory reports must be filed with the source documents.

If laboratory values from laboratory assessments that are not specified in the protocol and performed at the institution's local laboratory result in the need for significant intervention, lead to discontinuation of blinded trial drug, or are considered unanticipated or more severe than expected for the patient's medical condition based on the Investigator's clinical judgment (e.g., are considered to be an AE or SAE), then the results must be recorded in the AE section of the eCRF.

8.3.4.3 Anti-Drug Antibodies (ADA)

Blood samples will be taken for ADA determination at Baseline, and on Day 28 and Day 90. Samples taken on Day 90 will only be analyzed in case of a positive result on Day 28. The date and time of blood collection will be documented in the eCRF.

In addition, patients with a positive result on Day 90 will have an additional sample taken at Day 180, and if still positive at Day 180, further blood sampling will be scheduled for Day 360 (+/- 30 days). As ADA data beyond Day 90 will be generated on an infrequent, individual case

basis, results will be reported separately outside of the eCRF, and date and time of blood collection will not be documented in the eCRF.

Immunological testing will be performed by a central laboratory by using an Enzyme Linked Immunosorbent Assay (ELISA) on a Meso Scale Discovery (MSD) platform. The assay is validated in human and septic serum samples.

If the patient discontinues the trial between Day 28 and Day 90, an ADA sample must preferably be drawn at the EOT visit.

8.3.4.4 Adverse Events

The definitions of AEs, TEAEs and SAEs can be found in Appendix 2.

The Investigator and any qualified designees are responsible for detecting, documenting, and reporting events that meet the definition of AEs, TEAEs or SAEs regardless of their relationship to trial drug, and remain responsible for following up AEs as described in Appendix 2. Each AE must be assessed for seriousness, causality and severity (using severity grades defined in Appendix 2).

It is recognized that the patient population in the ICU or intermediate care unit will experience a number of common aberrations in laboratory values, signs, and symptoms due to the severity of the underlying disease and the impact of standard therapies. These will not necessarily constitute an AE unless they require significant intervention, lead to discontinuation of blinded trial drug, or are considered unanticipated or more severe than expected for the patient's medical condition based on the Investigator's clinical judgment.

8.3.4.4.1 Time Period for Collecting AE and SAE Information

All AEs (including SAEs), regardless of their suspected relationship to trial drug, will be collected and assessed from the time the patient signs the ICF up to and including Day 28. Information to be recorded on the AE page of the eCRF includes:

- event term
- time of onset
- Investigator-specified assessment of severity and relationship to trial drug
- date of resolution of the event
- seriousness
- any required treatment or evaluations
- outcome

Adverse events resulting from concurrent illnesses, reactions to concurrent illnesses, reactions to concurrent medications, or progression of disease states must also be reported if judged by the Investigator to be more severe than expected for the patient's medical condition. The Medical Dictionary for Regulatory Activities (MedDRA) will be used to code all AEs.

Medical occurrences that begin before the start of the patient's consent to participate in this trial and that continue after signing of informed consent will be recorded on the Medical History section of the eCRF.

After Day 28, only AEs that meet the SAE criteria and that the Investigator considers possibly, probably, or definitely related to the trial drug should be reported to the Sponsor or designee.

All SAEs must be recorded and reported to the Sponsor or designee within 24 hours after site staff first learns about the event, as indicated in Appendix 2. The Investigator will submit any updated SAE data to the Sponsor or designee within 24 hours of receipt of the information.

Overview of methods for recording, evaluating, and assessing causality of AEs and SAEs and procedures for completing and submitting SAE reports are provided in Appendix 2 and detailed in this trial's safety reporting documentation.

8.3.4.4.2 Follow-up of AEs and SAEs

All AEs must be followed to Day 28 (inclusive) or 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 if prior to Day 28. Ongoing SAEs on Day 28 will be 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 patient is LTFU (as defined in Section 7.3). Further information on follow-up procedures is given in Appendix 2.

8.3.4.4.3 Regulatory Reporting Requirements for SAE

- Prompt notification of an SAE (within 24 hours of becoming aware of the event, see Appendix 2) by the Investigator to the Sponsor or designee is essential so that legal obligations and ethical responsibilities towards the safety of patients are met.
- Any AE that is serious, associated with the use of the trial drug, and unexpected (SUSAR) has additional reporting requirements, as described in Appendix 2.
- The Sponsor or its designee will notify the Investigators in a timely fashion of relevant information about SUSARs that could adversely affect the safety of patients. Follow-up information may be submitted if necessary.
- The Sponsor will comply with country-specific regulatory requirements relating to safety reporting to the regulatory authority, IRB/IEC, and Investigators.

8.3.4.4.4 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 trial drug. Such antibodies could interfere with the ability to have a successful pregnancy. While development of ADAs has not been seen to date in human studies, the safety experience with this drug is limited to 219 subjects who received trial 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 will be excluded from the trial (see Section 5.2). Any pregnancy that occurs during trial participation must be reported to the Sponsor or its designee within 24 hours as described in Appendix 2.

The patient will be followed to determine the outcome of the pregnancy (including spontaneous or induced abortion, normal or complicated delivery, stillbirth, and normal offspring or congenital abnormality) and status of mother and child, even if the patient was discontinued from the trial. All outcomes of pregnancy must be reported by the Investigator to the Sponsor or its designee as described in Appendix 2 within 30 days after gaining this knowledge.

8.3.4.4.5 Laboratory Analyses

Any abnormal laboratory test results (hematology or clinical chemistry), including those that worsen from baseline, considered by the Investigator to be clinically significant and not related to progression of the underlying disease (unless judged by the Investigator to be more severe than expected for the patient's condition) are to be recorded as (S)AEs.

8.3.5 Others

8.3.5.1 Pharmacokinetics

Blood will be collected for (population) PK measurements on Day 3, Day 4, Day 5, and Day 7. On Day 3, 3 mL blood will be collected immediately prior to start of the infusion of the trial drug and approximately 2.5 – 3.5 hours after start of the infusion. On Day 4, Day 5 and Day 7, blood samples can be taken at any time. Samples will be analyzed by a central laboratory. Processing, storage and shipment of the samples are described in detail in the Laboratory Manual. Time and date of sample collection must be recorded in the eCRF. If sample collection and processing during off-hours is not practically feasible, it will not be considered a protocol deviation if the time critical samples on Day 3 cannot be performed in accordance with the specified windows.

8.4 Medication Errors and Treatment of Overdose

In healthy volunteers, the maximum administered single dose that did not result in any SAEs was 3.2 mg/kg (2,000 U/kg). The maximum multiple dose that was administered to healthy volunteers was 1.6 mg/kg (1,000 U/kg) per day for three days without any SAEs.

No specific treatment is available for medication errors including overdose (i.e., no reason to unblind) and there are no indications that specific symptoms will occur. If a medication error occurs, as a general rule, supportive care and treatment of symptoms must be provided.

8.5 Genetics

Pharmacogenomics are not evaluated in this trial.

9 Statistical Considerations

Statistical analyses will be performed by the Sponsor or its designee. Further details of the statistical analysis (including shells for tables, figures and listings) will be described in an SAP, which will be finalized prior to first unblinding of the trial database, i.e., prior to the database snapshot for the first safety data review. A separate DMC SAP will describe the analyses to be performed for the safety data reviews and interim analyses. The DMC SAP will be finalized before the first safety data review.

Database snapshots will be used for safety data reviews and interim analyses.

A further database snapshot will be executed after all patients in the main trial population have reached Day 28. Analysis of the primary endpoint will be performed on these data by the unblinded statistician and the result, i.e., whether the primary endpoint was met, presented to the Sponsor in a blinded manner. No further analyses will be performed at this time.

An interim lock will take place after all patients in the main trial population have reached Day 90. Endpoints defined up to and including Day 90 will be analyzed and the results used to start the preparation of the Clinical Trial Report (CTR). All personnel involved in patient care, data

collection or data monitoring will remain blinded to the individual patient's treatment allocation to minimize bias of ongoing data collection.

The final DBL will take place after all patients have completed the trial (i.e., all patients have completed Day 180 or have withdrawn/are LTFU prior to Day 180).

If patients in the 'moderate' CKD population have not completed the trial at the time of the interim lock at Day 90 and/or final DBL at Day 180 for patients in the main trial population, a separate interim lock at Day 90 and/or final DBL at Day 180 may be performed for patients in the 'moderate' CKD population in order for the analysis of data from patients in the main trial population to commence. If the COVID-19 population completes the trial at any time prior to the final DBL, an interim lock may be performed for these patients only. Full details of interim locks and the final DBL(s) will be documented.

Analysis sets will be defined for each population of patients, as appropriate. All statistical analyses of efficacy endpoints will be performed on the modified Intent-to-Treat (mITT) analysis sets. All statistical analyses of safety endpoints will be performed on the safety analysis sets.

Analysis of data from each population will be performed and presented separately. Formal analyses (incl. interim analyses), hypothesis testing and descriptive analyses will be performed on data from the main trial population, whereas only descriptive statistics (including an estimate of the treatment effect, two-sided 95% confidence intervals and one-sided p-value) will be presented for the 'moderate' CKD population and for the COVID-19 population.

9.1 Analysis Sets

The following analysis sets are defined for each population of patients, as appropriate:

Intent-to-Treat (ITT) Set: All patients in the population who are randomly assigned to a trial drug. Patients will be analyzed according to the treatment to which they were randomly assigned, regardless of whether they received what was assigned. Strata as entered in the IRT will be used.

modified Intent-to-Treat (mITT) Set: All patients in the population who are randomly assigned to a trial drug and for whom administration of trial drug was started. Patients will be analyzed according to the treatment to which they were randomly assigned, regardless of whether they received what was assigned. Strata as entered in the IRT will be used. The mITT set will be considered as the primary analysis set for the efficacy analyses.

Per protocol (PP) set: All mITT patients in the main trial population without any major protocol deviations. Major protocol deviations will be determined at data review meetings held before the interim lock and unblinding. The PP set will only be defined for the main trial population.

Safety Set: All patients in the population who received any trial drug. All safety analyses will be based on the Safety Set, with patients analyzed according to the trial drug they actually received.

PK Set: All patients who are randomly assigned to a trial drug, received at least one dose of the trial drug and have at least one PK result. The PK analyses will be based on this set, with patients analyzed according to the trial drug they actually received.

9.2 Statistical Analyses

9.2.1 Efficacy Analyses

Analysis of the data from each population will be presented separately. Formal analyses (incl. interim analyses), hypothesis testing and descriptive analyses will be performed on data from the

main trial population, whereas only descriptive statistics (including an estimate of the treatment effect, two-sided 95% confidence intervals and one-sided p-value) will be presented for the 'moderate' CKD and for the COVID-19 population.

9.2.1.1 Analysis of the Primary Efficacy Endpoint

The primary efficacy endpoint is "28-day all-cause mortality", defined as the probability to die (from any cause) up to and including Day 28. The primary analysis will be based on a logistic regression model with site as a random effect, treatment as a fixed effect, mSOFA score as an ordered categorical variable and eGFR at Baseline as the single continuous covariate.

9.2.1.1.1 Statistical Analysis

The treatment effect of recAP will be expressed as an adjusted odds ratio together with the 95% confidence interval (CI). The null hypothesis is that the odds ratio is equal to unity and this will be tested against the one-sided alternative hypothesis that the odds ratio is less than unity at an overall one-sided 0.025 significance level. The ordered categorical variable for mSOFA will break the scale at 9 (≤9 versus >9) to comply with the stratified randomization, but further cuts will be considered at the blind review stage, one below 9 and one above 9, based on approximately equal increments in expected mortality on the logit scale for the two treatment groups combined.

It is expected that there will be only a small amount of missing data on Day 28 mortality. Nonetheless, to account for such data, the primary analysis (including the interim looks) will utilize multiple imputation based on a logistic regression model fitted to the group of patients with data on the primary endpoint. Additional aspects of the modelling, imputation and inference will be specified in the SAP.

The following sensitivity analyses will be conducted for the primary endpoint:

- Logistic regression as for the primary analysis with the additional covariates APACHE II score and time from fulfilling both inclusion criteria 3 and 4 to time to treatment (hours). This analysis assesses the robustness of the findings to imbalances in those two covariates.
- Day 28 all-cause mortality obtained based on Kaplan-Meier (KM) curves for time to death up to Day 28. The KM curves will be compiled separately for the mSOFA categories (≤9 versus >9) and treatment differences in Day 28 survival rates combined using a stratified z-test. In this analysis, patients with unknown vital status who withdraw prior to Day 28 will be censored at the time of withdrawal. Patients ongoing in the trial who are known to be alive beyond Day 28 at the time of the analysis will be censored at Day 28. Patients LTFU prior to Day 28 will be censored at their last date known to be alive. This analysis assesses the impact of missing data on survival status on Day 28.
- A tipping point analysis in which all recAP patients with missing data on survival status on Day 28 will be considered as being dead, while all placebo patients with missing data on survival status on Day 28 will be considered as being alive, and all possible combinations of missing data between these two extremes will be considered.

9.2.1.1.2 Subgroup Analyses

Subgroup analyses (including forest plots of subgroup-specific 2-sided 95% CIs for the difference in 28-day all-cause mortality probabilities) will be performed for subgroups as defined in the SAP. As a minimum, the following subgroups will be analyzed:

- Region (US/non-US)
- Age (<65, ≥65 years)
- Gender

These analyses will use the same model as for the analysis of the primary endpoint in the mITT population, restricted to the respective subgroups.

9.2.1.2 Analysis of Secondary Efficacy Endpoints

Multiplicity for the analysis of secondary efficacy endpoints will be controlled by:

- initiating the test procedures for secondary efficacy endpoints only if the null hypothesis for the primary efficacy endpoint has been rejected, and
- using sequential conditional testing of null hypotheses for secondary efficacy endpoints in the order as indicated in Section 3; the nominal 1-sided significance level used within the sequential testing will be at the same alpha allocated to the primary endpoint at the time of the analysis.

9.2.1.2.1 Major Adverse Kidney Events (MAKE) 90

MAKE 90 is defined as dead by Day 90 *or* on RRT at Day 90 *or* ≥25% decline in eGFR on both Day 28 and Day 90 relative to the known or assumed pre-AKI reference level. The primary analysis will be based on a logistic regression model with site as a random effect, treatment as a fixed effect, mSOFA score as an ordered categorical variable, and pre-AKI reference eGFR as the single continuous covariate. The presentation of the results and the handling of missing data will be as described for the primary endpoint.

The following sensitivity analysis will be conducted for this endpoint:

- Logistic regression as for the primary analysis with the additional covariates APACHE II score and time from fulfilling both inclusion criteria 3 and 4 to time of treatment (hours). This analysis assesses the robustness of the findings to imbalances in those two covariates.
- MAKE 90 excluding death due to COVID-19 infection following initial hospital discharge will be analyzed as per the primary analysis logistic regression model which includes site as a random effect, treatment as a fixed effect, mSOFA score as an ordered categorical variable, and pre-AKI reference eGFR as the single continuous covariate.

9.2.1.2.2 Days Alive and Free of Organ Support Through Day 28

Days alive and free of organ support through Day 28 is to be defined as days alive with no MV, RRT, vasopressors or inotropes and with death within 28 days counting as zero days.

It is likely that these data will have distributions in each of the two treatment groups that are non-Normal and to deal with this the primary analysis will utilize a non-parametric method. The

method for assessing statistical significance will be a re-randomization test comparing the treatment median values for days alive and free of organ support, respecting randomization according to site and mSOFA score. 95% CIs for the difference in the medians will be constructed to aid interpretation.

The following sensitivity analysis will be conducted for this endpoint:

- Analysis will be repeated for the subset of patients alive on Day 28 in order to separate out the effect of mortality during the first 28 days.

9.2.1.2.3 Days Alive and Out of the ICU Through Day 28

In the analysis of this endpoint, death through Day 28 will count as zero days and the analysis will be as for days alive and free of organ support through Day 28 (see Section 9.2.1.2.2).

9.2.1.2.4 Time to Death Through Day 90

All-cause mortality up to and including Day 90 will be based on time from date of randomization to date of death. Patients with unknown vital status who withdraw prior to Day 90 will be censored at the time of withdrawal. Patients known to be alive on Day 90 will be censored on Day 90 and patients LTFU prior to Day 90 will be censored at their last date known to be alive.

Survival curves for each trial drug group will be estimated by the KM method. Median survival and corresponding 2-sided 95% CIs will be computed by the Brookmeyer and Crowley method[51].

The primary analysis of this secondary endpoint will be based on the Cox proportional hazards model with site as a random effect, treatment as a fixed effect, mSOFA score as an ordered categorical variable and eGFR at Baseline as the single continuous covariate as for the primary endpoint analysis. The treatment effect will be expressed as a hazard ratio (HR) together with a 95% two-sided CI.

The following sensitivity analyses will be conducted for this endpoint:

- Cox proportional hazards model as for the primary analysis with the additional covariates APACHE II score and time from fulfilling both inclusion criteria 3 and 4 to time of treatment (hours). This analysis assesses the robustness of the findings to imbalances in those two covariates.
- Day 90 all-cause mortality obtained based on KM curves for time to death up to Day 90. The KM curves will be compiled separately for the mSOFA score categories ≤9 and >9 subgroups and treatment differences in the Day 90 survival rates combined using a stratified z-test. This analysis makes no assumptions about the proportionality of the hazard rates and assesses the robustness of the findings to that assumption.
- Mortality at Day 90 excluding death due to COVID-19 infection following initial hospital discharge will be analyzed as per the primary analysis Cox proportional hazards model with site as a random effect, treatment as a fixed effect, mSOFA score as an ordered categorical variable and eGFR at Baseline as the single continuous covariate.

9.2.1.3 Tertiary/Exploratory Endpoints

Tertiary efficacy endpoints will be summarized by trial drug group. Tertiary endpoints will be viewed as exploratory and, if applicable, a nominal 1-sided significance level of 0.025 will be used and/or 2-sided 95% CIs will be provided.

As a minimum, the following tertiary endpoints will be evaluated:

- Time to death through Day 180.
- Change in total and individual organ failure scores through Day 7 (based on the mSOFA scores).
- Days alive and free of RRT through Day 28 (with death within 28 days counting as zero days).
- MAKE 28: dead by Day 28 *or* on RRT at Day 28 *or* ≥25% decline in eGFR on both Day 7/ICU discharge (whichever comes first) and Day 28 relative to the known or assumed pre-AKI level.
- Patients alive and free of AKI on Day 7/ICU discharge (whichever comes first) and on Day 28.
- Patients alive and free of new onset CKD or worsening of CKD (defined as any increase in CKD Stage) on Day 90.
- Days alive and free of vasopressor and inotropes through Day 28 (with death within 28 days counting as zero days).
- Days alive and free of MV through Day 28 (with death within 28 days counting as zero days).
- Days alive and out of the hospital through Day 90 (with death within 90 days counting as zero days).
- Incidence of at least one rehospitalization at any hospital through Day 90.
- Change in index values, QALY and VAS score based on the EQ-5D-5L questionnaire at Day 28, Day 90 and Day 180.
- The urinary levels of purines through Day 4 at selected sites.

Additional tertiary endpoints and further details regarding the statistical analysis of these endpoints will be given in the SAP.

9.2.1.4 Safety Endpoints

Safety parameters will be evaluated on the Safety Set.

Incidence of AEs, SAEs and TEAEs categorized by MedDRA System Organ Class (SOC) and Preferred Term (PT) will be summarized by trial drug group. Adverse event seriousness, severity, relationship to trial drug, and whether leading to discontinuation of trial drug will also be displayed in summaries and listings.

Local laboratory assessments will be summarized using descriptive statistics by trial drug group. Changes from baseline laboratory assessments will be summarized per trial drug group.

Anti-recAP antibodies results will be listed, including the results of the screening test and, if needed, the results of the confirmatory test and titer determination per dose group.

9.2.2 Other Analyses

Information from any additional, baseline, or screening assessments (e.g., disposition of patients, demographics, medical history, site of infection and pathogen, and APACHE II score) will be summarized using descriptive statistics for continuous variables, or frequency counts and percentages for categorical variables.

9.2.3 Population Pharmacokinetics

A population PK analysis of plasma concentration-time data will be performed using non-linear mixed-effects modeling. Data from this trial may be combined with data from the Phase 1 trial in healthy adult volunteers and/or Phase 2 trial (STOP-AKI) in SA-AKI patients and included in an integrated PK analysis. The structural model will contain clearance and volume of distribution as fixed-effect parameters. The inter-patient variability in the parameter estimates and the random residual error in the data will be estimated with an appropriate 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.

9.3 Interim Analyses

A maximum of four unblinded interim analyses will be conducted. Complete details of the interim analyses are provided in Appendix 3.

To maintain the blind and trial integrity, unblinded interim analysis outputs will be generated by a separate unblinded Biostatistics and Statistical Programming team not otherwise involved in the trial conduct. These unblinded interim results will exclusively be provided to the trial's DMC for review. The DMC will operate according to an approved DMC Charter and the DMC Chair will provide written recommendations on trial continuation or discontinuation to the Sponsor.

At the first interim analysis, the trial may be stopped for futility. At subsequent interim analyses, the trial may be stopped for futility or for early success (demonstration of superiority of recAP over placebo on 28 days mortality).

Success

The Lan-DeMets approximation of the O'Brien-Fleming alpha spending function will be used to determine the critical values for declaring success at interim and final analyses. Table 8 shows the nominal 1-sided significance levels at interim and final analyses for declaring success if data from exactly 700, 850, 1,000 and 1,400 patients in the main trial population will be available. In case the actual patient numbers differ, then the nominal significance levels will be re-calculated by the Lan-DeMets approach.

Table 8: Nominal 1-Sided Significance Levels for Success at Interim and Final Analyses (per the Lan-DeMets Approximation of the O'Brien-Fleming Alpha Spending Function)

Number of Patients Complete	Nominal One-Sided *p*-value
700	0.0015
850	0.0036
1,000	0.0067
1,400	0.0224

This trial will be considered a success if the 1-sided p-value from the primary efficacy analysis model for the treatment term is lower than the respective nominal 1-sided significance level.

Should the trial be stopped for success at an interim analysis, then the analysis of the secondary endpoints within the sequential testing will proceed using the nominal 1-sided significance level as allocated to the primary endpoint at the time of the analysis.

Early Futility

The predictive probability of success at the main trial population's maximum sample size (PP_{max}) of 1,400 patients will be used to determine if the trial should stop early for futility. This predictive probability calculation combines the knowledge of the treatment effect observed in the trial with the uncertainty of the future data not yet observed. At the first interim analysis, the trial may stop for futility if the predictive probability of trial success is less than 15%. At each subsequent interim analysis, the trial may stop for futility if the predictive probability of trial success is less than 5%. Futility stopping in this trial is considered to be non-binding.

9.4 Sample Size Determination

For this complex trial design, the operating characteristics (Type I error probability and power) are derived via simulations for a PP_{max} of 1,400 patients in the main trial population, and the group-sequential design described in Section 9.3.

Simulation Assumptions

Several underlying truths for the control and treatment in terms of the 28-day all-cause mortality probability in each trial drug group were defined. Operating characteristics are based on 10,000 simulations per scenario. Drop-outs were not included in the simulations as the number of dropouts is expected to be very low. Simulations include simulation of all interim analyses with early success and early futility decisions in place. Simulations were performed using FACTS 6.2 (Berry Consultants, Austin TX).

Operating Characteristics

Operating characteristics are reported in Table 9. Total probability of trial success, mean sample size, probability of early success, and probability of trial futility are shown. 28-day all-cause mortality probability in the placebo treatment group was set at 35% across all scenarios, which

was the observed 28-day mortality in the sub-group of patients with an eGFR <60 mL/min in STOP-AKI. Different treatment effects from a 0% to a 15% absolute improvement were explored. The scenario where 28-day all-cause mortality probabilities are the same (0% improvement) for recAP and placebo treatment groups represents the null hypothesis. The probability of trial success in that scenario can be interpreted as the trial's simulated Type I error probability.

Table 9: Operating Characteristics with Maximum Sample Size of 1,400 and Chosen Group-sequential Design

Operating Characteristics						
Control Mortality Rate	**Treatment Mortality Rate**	**Absolute Improvement**	**Probability of Success (Power)**	**Mean N**	**Probability Early Success**	**Probability Early Futility**
35%	35%	0%	0.022	696	0.006	0.856
35%	33%	2%	0.103	857	0.038	0.663
35%	31%	4%	0.310	1005	0.132	0.425
35%	30%	5%	0.461	1054	0.216	0.308
35%	29%	6%	0.616	1067	0.333	0.211
35%	28%	7%	0.753	1050	0.472	0.136
35%	27%	8%	0.856	1010	0.614	0.082
35%	26%	9%	0.925	960	0.741	0.046
35%	25%	10%	0.966	906	0.843	0.023
35%	20%	15%	1.000	757	0.998	0.000

In the scenario where both the control and the treatment are equal at a 35% 28-day all-cause mortality rate, the overall simulated 1-sided Type I error probability is 0.022. In this scenario of 0% improvement, there is an 86% probability of early futility and the mean sample size is 696 patients in the main trial population. If there is a 6% absolute treatment benefit, this trial has 61.6% power with a 33.3% probability of early success and a mean sample size of 1,067 patients. If there is an 8% absolute treatment benefit, this trial has 85.6% power and the mean sample size is decreased to 1,010 patients due to an increase in the probability of early success. Additional operating characteristics assuming different control rates are provided in Appendix 3.

No formal sample size determination based on power calculation was performed for the 'moderate' CKD population or the COVID-19 population as no formal hypothesis testing is planned for these two populations. Up to approximately 100 patients in each population will be enrolled, which is considered adequate for the exploratory nature of these populations.

10 Supporting Documentation and Operational Considerations

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

10.1.1 Regulatory and Ethical Considerations

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 trial or increasing the risk to patients.

- This trial will be conducted in accordance with the protocol and:
  - Consensus ethical principles derived from international guidelines including the Declaration of Helsinki and Council for International Organizations of Medical Sciences (CIOMS) International Ethical Guidelines.
  - Applicable International Conference on Harmonisation (ICH) GCP Guidelines.
  - Applicable laws and regulations.
- Federal regulations and the ICH E6(R2) guidelines require that approval be obtained from an IRB/ IEC before participation of human patients in research studies. The protocol, substantial protocol amendments, ICF, IB, and other relevant documents (e.g., any other written information regarding this trial to be provided to the patient or the patient's legal representative) must be submitted to an IRB/IEC by the Investigator and reviewed and approved by the IRB/IEC prior to being used in the trial. Documentation of all IRB/IEC approvals and of the IRB/IEC compliance with ICH E6(R2) will be maintained by the site and will be available for review by the Sponsor or its designee. All IRB/IEC approvals must 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, IRB/IEC composition at the meeting and the date approval or a favorable opinion was granted.
- Any substantial amendments to the protocol will require IEC/IRB approval before implementation of changes made to the trial design, except for changes necessary to eliminate an immediate hazard to trial patients.
- The Investigator will be responsible for the following:
  - Providing written summaries of the progress and status of the trial to the IRB/IEC annually or more frequently in accordance with the requirements, policies, and procedures established by the IRB/IEC.
  - Overall conduct of the trial at the site and adherence to requirements of 21 Code of Federal Regulations (CFR), ICH GCP guidelines, the IRB/IEC, European regulation 536/2014 for clinical studies (if applicable), and all other applicable local regulations.
  - Submitting reports of SAEs according to the timeline and method outlined in the protocol.

10.1.2 Financial Disclosure

Investigators and sub-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 or its designee a commitment to promptly update this information if any relevant changes occur during the course of the trial and for one year following completion of the trial.

Neither the Sponsor nor its designee 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 its designee is financially responsible for further treatment of the patient's disease.

10.1.3 Informed Consent Process

It is anticipated by the very nature of the trial 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 disease severity, sedation or unconscious state. In a situation where a patient is unable to provide consent, the patient's legal 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 the consent procedure has been approved by the IRB/IEC or national authorities, whatever is applicable in the specific country. 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 with the most current version of the ICF(s) as soon as they are able.

If all inclusion criteria and none of the exclusion criteria that can be assessed in a non-invasive manner is met, the attending physician or a trial team member will ask the patient or his/her legal representative, an independent physician or any other person allowed per local legal requirements to consent on behalf of the patient, if the patient is willing to participate in the trial. It must be explained that patient's participation in the trial is voluntary. If they agree, patients (or his/her legal representative, or independent physician, or other according to local regulatory requirements) will be informed verbally about the trial schedule and potential risks. They will also receive written information on the trial. Sufficient time will be allowed to read the information and to ask questions. A patient is not allowed to enter the trial if he/she (or his/her legal representative) has not understood the written and verbal information provided and/or without a signed and dated ICF. Patients (or their legal representative) will have the opportunity to have any questions addressed before signing the ICF. The person who obtains the consent must address all questions raised by the patient or their legal representative. The person obtaining consent will also sign and date (incl. time) the ICF (at the same date and time as the patient or legal representative). A copy of the written information and the signed ICF will be provided to the patient (or his/her legal representative), whereas the original version will be retained by the Investigator. The ICF will meet the requirements of 21 CFR 50, local regulations, ICH guidelines, Health Insurance Portability and Accountability Act (HIPAA) requirements, where applicable, and of the IRB/IEC or trial center.

The medical records must include a statement that written informed consent was obtained before the patient was enrolled in the trial and the date and time the written consent was obtained.

Patients who are currently enrolled and have not yet completed the trial need to provide re-consent if a new version of the ICF has been approved.

It is the responsibility of the Investigator to ensure that the patient meets the trial enrollment criteria.

For centers in the European Union (EU): The explicit wish of a mentally incapacitated adult, who is capable of forming an opinion and assessing the trial information, to refuse participation in or to be withdrawn from the trial at any time will be respected by the Investigator.

If a protocol amendment is required, the ICF may need to be revised to reflect the changes to the protocol. If the ICF is revised, it must be reviewed and approved by the appropriate IEC/IRB and signed by all patients who are currently enrolled and have not yet completed the trial and those patients who will be subsequently enrolled in the trial.

10.1.4 Data Protection

- Patients will be assigned a unique patient identification number via the IRT system. Any patient records or datasets that are transferred to the Sponsor will contain this identifier only; patient names and any information which would make the patient identifiable will not be transferred. All laboratory specimens, evaluation forms, reports, and other records will be identified in a manner designed to maintain patient confidentiality.
- The patient must be informed that his/her personal trial-related data will be used by the Sponsor in accordance with local data protection laws as stated in the ICF. The level of disclosure must also be explained to the patient as well as his/her rights including the right to access or erase his/her personal data.
- The patient must be informed that his/her medical records may be reviewed by Clinical Quality Assurance auditors or other authorized personnel appointed by the Sponsor, by appropriate IRB/IEC members, and by inspectors from regulatory authorities.
- In the event the Day 28 and/or Day 90 visit is conducted as a home visit, the patient must be informed that his/her home address may be shared with the courier company which may ship the blood sample collected.
- All records will be kept in a secure storage area with limited access.
- The Investigator and Institution will, at all times, comply with applicable data protection laws.

10.1.5 Committees Structure

A Trial Steering Committee (TSC) will be established and will consist of non-participating experts in the field of SA-AKI, National Coordinators of participating countries and Sponsor representatives (non-voting members). The TSC will provide leadership and oversee the overall trial conduct in a blinded fashion and make recommendations to AM-Pharma regarding trial-related decisions including those based on recommendations from the DMC. The Trial Steering Committee will follow an agreed Trial Steering Committee Charter.

Likewise, a DMC, consisting of independent critical care and nephrology experts not otherwise involved in the trial and a statistician, will be established for the trial. The DMC will evaluate

safety (SAEs) at regular intervals throughout the trial. In addition, the DMC will review the interim analyses reports and notify the Sponsor and the Trial Steering Committee in case a futility or success threshold is reached. The DMC will follow an agreed charter and will provide written communication to the Sponsor and the Trial Steering Committee on its recommendation on trial continuation, discontinuation or proposed modifications.

Details on the DMC will be described in the DMC charter.

10.1.6 Dissemination of Clinical Trial Data

Investigators, employees or coworkers involved in this trial may not disclose nor use, for any purpose other than performance of the trial in accordance with this Protocol, any data, record, or other unpublished, confidential information disclosed to those individuals for the purpose of the trial. Prior written agreement from the Sponsor or its designee must be obtained for the disclosure of any said confidential information to other parties.

Irrespective of whether the trial is completed or prematurely terminated, the Sponsor will ensure that the CTRs are prepared and provided to the regulatory agency(ies) as required by the applicable regulatory requirement(s). The Sponsor will also ensure that the CTRs in marketing applications meet the standards of the ICH E6(R2): Structure and content of CTRs.

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

Upon completion of the CTR, the Sponsor will provide the Investigator with the full summary of the trial results. The Investigator is encouraged to share the summary results with the trial patients, as appropriate. The trial results will be posted on publicly available clinical trial registers in accordance with the requirements.

10.1.7 Data Collection, Quality Assurance, and Management

- Quality control for this trial will be performed in compliance with the contract research organization (CRO)'s SOPs. The ICH E6(R2) and CFR audits for this trial will be performed in compliance with CRO's SOPs. The Sponsor may conduct additional audits either directly or through a third-party. Such audits will be performed in compliance with the Sponsor / third-party SOP's.
- Clinical data will be captured using electronic data capture (EDC) technology unless otherwise specified in this document. Once the eCRF clinical data have been submitted to the central server at the independent data center, corrections to the data fields will be captured in an audit trail. The reason for change, the name of the person who performed the change, together with the time and date will be logged to provide an audit trail.
- The format and content of the eCRF will be approved by the Sponsor or its designee prior to the start of the trial. Clinical data that are not captured via the eCRF (e.g., data from central laboratory), will be integrated with the eCRF data. The Investigator is responsible for timely entry of data following each patient's visit and for verifying regularly that data entries are accurate and correct by electronically signing the eCRF pages. The sites will promptly address any reported discrepancies.

- All eCRF fields are to be completed. If an item is not available or is not applicable, this fact must be indicated. Blank spaces must not be present unless otherwise indicated.
- The specific instructions for data entry and query resolution in the EDC system/eCRF will be provided to trial sites in an eCRF Completion Manual. In addition, site personnel will receive training on the EDC system/eCRF.
- Protocol deviations will be tracked by the Sponsor or its designee.
- Data that are required for this trial are to be recorded in the patient's medical notes/source documents and then entered into the eCRF by authorized trial personnel.
- The Investigator must provide direct access to the source documents to the Sponsor or its designee. Source data are defined as all information related to clinical findings, observations, or other activities in the trial, written down in original records or certified copies of original records. Source documentation supporting the eCRF data must indicate the patient's participation in the trial and must document the dates and details of trial procedures, AEs, other observations and patient status. It is the Investigator's responsibility to ensure the accuracy, completeness, clarity, and timeliness of the data reported in the patient's eCRF. The eCRF will not be considered the source document for any data.
- Risk based monitoring will be implemented for this trial. The Source data verification process and definition of key variables to be monitored, will be described in a trial-specific Monitoring Plan. Trial monitors will check data entered in the eCRF against source data on a regular basis to confirm that data entered by authorized site personnel are accurate, complete, and verifiable from source documents, to assure that the safety and rights of patients are being protected and that the trial is being conducted in accordance with the approved protocol and any other trial agreements e.g., ICH GCP, and all applicable regulatory requirements.
- If corrections are needed, the responsible monitor or data manager will raise a query in the EDC application. The appropriate staff at the trial site will answer queries sent to the Investigator in a timely manner. The name of the staff member responding to the query, and time and date stamp will be captured to provide an audit trail. Once all source data verification is complete and all queries are closed, the eCRF page will be locked. The Investigator is responsible for timely resolution of all queries.
- The Sponsor or designee is responsible for the data management of this trial including quality check of the data. Further details of the clinical data management process will be described in the Clinical Data Management Plan, which will be finalized prior to first patient, first visit (FPFV).
- Clinical data management will be performed in accordance with applicable CRO standards and data cleaning procedures to ensure the integrity of the data, e.g., resolving 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 World Health Organization (WHO) Drug Dictionary.

- After all data reviews and query resolutions are complete, the database will have a quality assurance check to ensure acceptability and completeness, including reconciliation of other databases (i.e., safety database, third-party database). The SAP must be approved and signed prior to unblinding of the trial database, i.e., prior to the database snapshot for the first safety data review. Analysis populations must be approved prior to unblinding of the individual analyses (safety data reviews, interim analyses and DBL). The data analysis sets will be a combination of the data in the EDC system and data from other sources (e.g., laboratory data).
- The Investigator must permit trial-related monitoring, audits, IRB/IEC review, and regulatory agency inspections and provide direct access to source data documents. In the event of an audit, the Investigator agrees to allow the Sponsor or its designee, or a regulatory agency (e.g., FDA or other regulatory agency) access to all trial records. The Investigator must promptly notify the Sponsor, or its designee of any audits scheduled by any regulatory authorities and promptly forward copies of any audit reports received to the Sponsor or its designee.
- Essential documents must be retained until at least two 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 two years have elapsed since the formal discontinuation of clinical development of the trial drug. These documents must 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. No records may be destroyed during the retention period without the written approval of the Sponsor. No records may be transferred to another location or party without written notification to the Sponsor.
- As part of the responsibilities assumed by participating in the trial, the Investigator agrees to maintain adequate patient records for the patients treated as part of the research under this protocol. The Investigator agrees to maintain accurate eCRFs and source documentation that supports the information entered in the eCRF as part of the case histories. These source documents may include laboratory reports, etc.
- After the Day 180 DBL, each trial site will receive all of their site-specific eCRF data as entered into the EDC system for the trial, including full discrepancy and audit history. Additionally, a copy of all of the trial site's data from the trial will be created and sent to the Sponsor for storage. The CRO will maintain a duplicate copy for their records. In all cases, patient initials will not be collected or transmitted to the Sponsor.

10.1.8 Source Documents

- Source documents provide evidence for the existence of the patient and substantiate the integrity of the data collected. Source documents are filed at the Investigator's site.

  Data entered in the eCRF that are transcribed from source documents must be consistent with the source documents or the discrepancies must be explained. The Investigator may need to request previous medical records or transfer records, depending on the trial. Also, current medical records must be available.

10.1.8.1 Investigator Documentation

Prior to beginning the trial, the Investigator will be asked to comply with ICH E6(R2) 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 (for US sites) and the EU equivalent form Investigator Information and Agreement (for Non-US sites), fully executed, and all updates on a new fully executed Form. 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 one year after the completion of the trial.
- IRB/IEC-approval of protocol-specific ICF and any other written information regarding this trial that is to be provided to the patient or legal representative.
- Laboratory certifications and normal ranges for any local laboratories used by the site, in accordance with 42 CFR 493.

10.1.9 Trial and Site Closure

Upon completion of the trial, the Investigator, where applicable, must inform the institution; the Investigator/institution must provide the IRB/IEC with a summary of the trial's outcome and the Sponsor must provide the regulatory authority(ies) with any reports required.

The Sponsor reserves the right to close the trial site or terminate the trial at any time for any reason at the sole discretion of the Sponsor. Trial sites will be closed upon trial completion. A trial site is considered closed when all required documents and trial supplies have been collected and a trial site closure visit has been performed.

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

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

- Failure of the Investigator to comply with the protocol, the requirements of the IRB/IEC or local health authorities, the Sponsor's procedures, or GCP guidelines.
- Inadequate recruitment of patients by the Investigator.
- Discontinuation of further trial drug development.
- Clinical or administrative reasons.

10.1.10 Records Retention

Refer to Section 10.1.7.

10.1.11 Publication Policy

- At the end of the trial, the data will be reported at scientific meetings and/or submitted and published in a scientific journal. For that purpose, publication will be coordinated by the Trial Steering Committee and the Sponsor.
- The Sponsor supports publication of the Trial results, regardless of the outcome. It is understood that all data related to the trial remains the sole and exclusive property of the Sponsor.
- Therefore, no data resulting from the trial will be presented or published in any form or media by the Institution, Investigator or research staff without the prior written consent of the Sponsor. Investigators should submit all manuscripts or abstracts to the Sponsor for its prior written approval at least 60 days prior to submission. In addition, as the trial is a multi-center clinical trial, no individual publications will be made until the first multi-center publication or presentation. Subsequent publications must reference the primary publication and all publications will acknowledge all trial sites.
- Authorship will be determined by mutual agreement between the Trial Steering Committee and potential co-authors based on relevant contribution in accordance with international scientific and ethical standards, including the International Committee of Medical Journal Editors authorship requirements.

10.1.12 Trial Administration

A list of personnel and organizations responsible for the conduct of the trial (details included in Table 10) will be provided by the Sponsor or its designee to the trial sites, as part of the Investigator Site File.

Table 10: Personnel and Organizations Responsible for Trial Conduct

Role	Name/Affiliation/Address
Sponsor	AM-Pharma B.V.
Sponsor Signatory and Sponsor Medical Officer	PPD
Contract Research Organization	Labcorp INC. 206 Carnegie Center, Princeton, New Jersey, 08540 USA
Trial Drug Manufacturing Facilities	
Manufacturing, Preparation and Dispensing:	Pfizer Manufacturing Belgium N.V Rijksweg 12 B-2870 Puurs Belgium Phone: +32 38909211
Labeling, Secondary Packaging and Shipping:	Almac Clinical Services 9 Charlestown Road, Seagoe Industrial Estate, Craigavon BT63 5PW United Kingdom
Responsible party for batch release (QP certification):	AM-PHARMA B.V., WTC Utrecht Stadsplateau 6 3521 AZ Utrecht The Netherlands Tel: +31 (0) 30 228 9222

10.1.13 Protocol Approval and Amendment and Protocol Deviations

10.1.13.1 Protocol and Protocol Amendments

Before the start of the trial, the trial protocol and/or other relevant documents will be approved by the IEC/IRB/Competent Authorities, in accordance with local and international legal requirements. The Sponsor must ensure that all ethical and legal requirements have been met before the first patient is enrolled in the trial.

This protocol is to be followed exactly. To alter the protocol, amendments must be written by the Sponsor or its designee and, if appropriate, receive IRB/IEC/Competent Authority approval prior to implementation (as locally required), except for changes necessary to eliminate an immediate hazard to patients.

Following approval, if applicable, the protocol amendment(s) will be submitted to the investigational new drug (IND) under which the trial is being conducted.

Administrative changes (not affecting the patient benefit/risk ratio) e.g., change in Medical Monitor or contact information may be made without the need for a formal amendment. All amendments will be distributed to all protocol recipients, with appropriate instructions.

10.1.13.2 Protocol Deviations

Protocol deviations are changes or departure from the trial design or procedures defined in the protocol. Important PDs are a subset of protocol deviations that might significantly affect the completeness, accuracy, and/or reliability of the trial data or that might significantly affect a patient's rights, safety, or well-being.

Protocol deviations will be documented by the clinical monitor throughout the course of monitoring visits. Investigators will be notified in writing by the monitor of all protocol deviations.

A priori protocol deviation (protocol waivers) will not be granted.

10.1.13.3 Access to Source Data

All aspects of the trial will be carefully monitored, by the Sponsor or its designee, for compliance with applicable government regulation with respect to current ICH E6(R2) and current SOPs.

During the trial, in addition to maintaining necessary phone and letter contact, a monitor will make periodic site visits to review protocol compliance, compare eCRF entries and individual patient’s medical records, assess drug accountability, and ensure that the trial is being conducted according to pertinent regulatory requirements. eCRF entries will be verified with source documentation in accordance with the Monitoring Plan. The review of medical records will be performed in a manner to ensure that patient confidentiality is maintained at all times.

Checking of the eCRF entries for completeness and clarity, and cross-checking with source documents, will be required to monitor the progress of the trial. Moreover, regulatory authorities of certain countries, IRBs, IECs, and/or the Sponsor or its designee’s Clinical Quality Assurance Group may wish to carry out such source data checks during on-site audits or inspections. Direct access to source data will be required for these inspections and audits; they will be carried out

respecting data protection and medical confidentiality. The Investigator will provide the Sponsor or its designee with the necessary support for said inspections and audits.

10.2 Appendix 2: Adverse Events: Definitions and Procedures for Recording, Evaluating, Follow-up, and Reporting

Adverse Event Definition
• An adverse event (AE) is 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. NOTE: An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated).

Events <u>Meeting</u> the Adverse Event Definition
• Any abnormal laboratory test results (hematology, clinical chemistry, or urinalysis) or other safety assessments (e.g., electrocardiogram [ECG], radiological scans, vital signs measurements), including those that worsen from baseline, considered clinically significant in the medical and scientific judgment of the Investigator (i.e., not related to progression of underlying disease). • Exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency and/or intensity of the condition. • New conditions detected or diagnosed after study drug administration even though it may have been present before the start of the study. • Signs, symptoms, or the clinical sequelae of a suspected drug-drug interaction. • Signs, symptoms, or the clinical sequelae of a suspected overdose of either study drug or a concomitant medication. • "Lack of efficacy" or "failure of expected pharmacological action" per se will not be reported as an AE or SAE. Such instances will be captured in the efficacy assessments. However, the signs, symptoms, and/or clinical sequelae resulting from lack of efficacy will be reported as AE or SAE if they fulfill the definition of an AE or SAE.

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

Treatment Emergent Adverse Event Definition
• A treatment emergent adverse event (TEAE) is defined as any event not present before exposure to study drug or any event already present that worsens in either intensity or frequency after exposure to study drug up to 14 days after last drug exposure.

Definition of Serious Adverse Event

An SAE is defined as any untoward medical occurrence that, at any dose:
Results in death
Is immediately life-threatening The term 'life-threatening' in the definition of 'serious' refers to an event in which the patient was at risk of death at the time of the event. It does not refer to an event, which hypothetically might have caused death, if it were more severe.
Requires inpatient hospitalization or prolongation of existing hospitalization In general, hospitalization signifies that the patient has been detained (usually involving at least an overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or outpatient setting. Complications that occur during hospitalization are AE. If a complication prolongs hospitalization or fulfills any other serious criteria, the event is serious. When in doubt as to whether "hospitalization" occurred or was necessary, the AE must be considered serious. Hospitalization for elective treatment of a pre-existing condition that did not worsen from baseline is not considered an AE.
Results in persistent disability/incapacity • The term disability means a substantial disruption of a person's ability to conduct normal life functions.

- This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhea, influenza, and accidental trauma (e.g., sprained ankle) which may interfere with or prevent everyday life functions but do not constitute a substantial disruption.

Is a congenital anomaly/birth defect

Other situations

- 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 or scientific judgment must be exercised in deciding whether SAE reporting is appropriate in other situations such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient or may require medical or surgical intervention to prevent one of the other outcomes listed in the above definition. These events should usually be considered serious.

  Examples of such events include invasive or malignant cancers, intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse.

Recording and Follow-up of Adverse Events and Serious Adverse Event

AE and SAE Recording

- When an AE/SAE occurs, it is the responsibility of the Investigator to review all documentation (e.g., hospital progress notes, laboratory, and diagnostics reports) related to the event.
- The Investigator will then record all relevant AE/SAE information in accordance with this trial's safety reporting documentation.
- It is **not** acceptable for the Investigator to send photocopies of the patient's medical records to the Sponsor or its designee in lieu of completion of the AE/SAE documentation.
- There may be instances when copies of medical records for certain cases are requested by the Sponsor or its designee. In this case, all patient identifiers, with the exception of the patient number, will be blinded on the copies of the medical records before submission to the Sponsor or its designee.
- The Investigator will attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. In such cases, the diagnosis (not the individual signs/symptoms or related procedure), as well as indication for the procedure, will be documented as the AE/SAE.

Assessment of Severity

The severity of an AE refers to the extent to which an AE affects the patient's daily activities. The Investigator will make an assessment of severity for each AE and SAE reported during the study and assign it to one of the following categories:

- Mild: An event that is easily tolerated by the patient, causing minimal discomfort and not interfering with everyday activities.
- Moderate: An event that causes sufficient discomfort and interferes with normal everyday activities.
- Severe: An event that prevents normal everyday activities. An AE that is assessed as severe must not be confused with an SAE. Severe is a category utilized for rating the intensity of an event; and both AE and SAE can be assessed as severe.

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

When changes in the severity of an AE occur more frequently than once a day, the maximum severity for the event must 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 must be documented to allow an assessment of the duration of the event at each level of intensity to be performed.

Assessment of Causality

- The Investigator is obligated to assess the relationship between study drug and each occurrence of each AE/SAE.
- A "reasonable possibility" of a relationship conveys that there are facts, evidence, and/or arguments to suggest a causal relationship, rather than a relationship cannot be ruled out.
    - The Investigator will use clinical judgment to determine the relationship.
    - Alternative causes, such as underlying disease(s), concomitant therapy, and other risk factors, as well as the temporal relationship of the event to study drug administration will be considered and investigated.
    - For each AE/SAE, the Investigator **must** document in the medical notes that he/she has reviewed the AE/SAE and has provided an assessment of causality.
    - There may be situations in which an SAE has occurred, and the Investigator has minimal information to include in the initial report to the Sponsor or its designee. However, **it is very important that the Investigator always make an assessment of causality for every event before the initial transmission of the SAE data to the Sponsor or its designee.**

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

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

- 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 study drug administration exists and, based upon the known pharmacological action of the study drug, known or previously reported adverse reactions to the study 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 study 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.

Pregnancy

- If a pregnancy is discovered during treatment with trial drug, trial drug must immediately be discontinued.
- While pregnancy itself is not considered to be an AE or SAE, any complication of pregnancy will be reported as an AE or SAE.
- Hospitalization for normal delivery of a healthy newborn should not be considered an SAE.
- Spontaneous abortion, ectopic pregnancy, stillbirth, neonatal death, or congenital anomaly are always considered to be an SAE and will be reported as such.

- Any SAE that occurs during pregnancy (including SAEs occurring after last administration of study drug) must be recorded and reported within 24 hours in accordance with this trial's safety reporting documentation. Any SAE that is not pregnancy-related but that occurs 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, probably, or definitely related to the study drug, must be reported to the Sponsor or its designee within 24 hours after site staff first learns about the event.

Follow-up of Adverse Event and Serious Adverse Event

- All AEs must be reported in detail on the appropriate page of the eCRF and followed to Day 28 (inclusive) or 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 if before Day 28.
- Ongoing SAEs on Day 28 will be 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 patient is lost to follow-up (LTFU).
- The Investigator is obligated to perform or arrange for the conduct of supplemental measurements and/or evaluations as medically indicated or as requested by the Sponsor or its designee to elucidate the nature and/or causality of the AE or SAE as fully as possible. This may include additional laboratory tests or investigations, histopathological examinations, or consultation with other health care professionals.
- If a patient dies during participation in the study or during a recognized follow-up period, the Investigator will provide the Sponsor or its designee with a copy of any post-mortem findings including histopathology.
- New or updated information will be recorded in the originally completed eCRF.
- The Investigator will submit any updated SAE data to the Sponsor or its designee within 24 hours of receipt of the information.

Reporting of Serious Adverse Event to the Sponsor or its designee

Serious Adverse Event Reporting to the Sponsor or its designee via Electronic Data Collection Tool

- The Investigator must report any SAEs to the Sponsor or its designee within 24 hours after site staff first learns about the event.
- All SAEs will be recorded from signing of informed consent until Day 28 regardless of their relationship to study drug. After Day 28, only SAEs that the Investigator considers possibly, probably, or definitely related to the study drug will be reported to the Sponsor or designee.

- When reporting an SAE, keep in mind that the event term should ideally be the diagnosis of the event based on signs, symptoms, and/or other clinical information. Causality assessment should also be provided.
- The Sponsor or its designee will review each SAE report and the Sponsor, or its designee will evaluate the seriousness and the causal relationship of the event to study drug. In addition, the Sponsor or its designee will evaluate the expectedness according to the IB. Based on the Investigator and Sponsor's assessment of the event, a decision will be made concerning the need for further action. The Sponsor or its designee will be responsible for all information processing and reporting according to local legal requirements.
- Contacts and instructions for SAE reporting can be found in the trial's safety reporting documentation.

Suspected Unexpected Serious Adverse Reactions (SUSARs)

Any AE that is serious, associated with the use of the study drug, and unexpected, i.e., not listed in current IB as expected for this study (SUSAR), has additional reporting requirements, as described below.

- If the SUSAR is fatal or life-threatening, associated with study drug, and unexpected, regulatory authorities and IRBs/IECs will be notified within 7 calendar days after the Sponsor or its designee learns of the event. Additional follow-up (cause of death, autopsy report, and hospital report) information must be reported within an additional 8 days (15 days total).
- If the SUSAR is not fatal or life-threatening but is otherwise serious, associated with study drug, and unexpected, regulatory authorities and IRBs/IECs will be notified within 15 calendar days after the Sponsor or its designee learns of the event.

The Sponsor or its designee will notify the Investigators in a timely fashion of relevant information about SUSARs that could adversely affect the safety of patients. Follow-up information may be submitted if necessary.

The Sponsor will also provide annual safety updates to the regulatory authorities and IRBs/IECs responsible for the study. These updates will include information on SUSARs and other relevant safety findings.

10.3 Appendix 3: Statistical Design Report

Statistical Design Report for
A Phase III Trial of recAP in Sepsis Acquired Acute Kidney Injury

Submitted to AM Pharma
September 9, 2019

1.0 Introduction

This document outlines the statistical design for a clinical trial to compare the efficacy of recAP to placebo control for the treatment of sepsis-associated acute kidney injury in patients with low estimated glomerular filtration rate (eGFR). The purpose of this document is to provide a description of the statistical design along with details of the statistical models, simulation assumptions, and simulation results. This trial will enroll a maximum of 1,400 patients randomized 1:1 to either recAP or placebo control. Interim analyses will take place during the conduct of the trial in order to stop early for success or for futility.

2.0 Primary Endpoint and Primary Analysis

The primary endpoint is the proportion of patients who die within 28 days from randomization. This endpoint will be estimated for each arm as a dichotomous proportion. Patients lost to follow-up within these 28 days will be imputed using multiple imputation techniques described in the protocol. The number of patients for whom the 28-day status will be unknown is expected to be very small.

The primary analysis will be a logistic regression model for 28-day mortality with site as a random effect, and fixed effects for modified Sequential Organ Failure Assessment (mSOFA) score, eGFR, and treatment assignment.

3.0 Interim Analyses

Interim analyses are planned according to information time, or the number of patients randomized and evaluable for the primary endpoint. Patients are considered evaluable for the primary endpoint if it has been at least 28 days since their date of randomization and their primary endpoint is known, or they are known to be lost to follow-up and will be included in the primary analysis according to the described missing data conventions. Interim analyses will take place when approximately 400, 700, 850, and 1000 patients are evaluable for the primary endpoint. At the first interim analysis, the study may be stopped for futility. At subsequent interim analyses, the study may be stopped for futility or for success. A DMC will monitor the study for safety and will review the unblinded interim analysis reports.

3.1 Early Success

We use the Lan DeMets approximation to an O'Brien-Fleming spending function to determine the critical values for declaring early trial success at each of the planned interim analyses. Table 1 reports the nominal one-sided *p*-values required at each analysis to declare success.

Table 1: Lan DeMets O'Brien-Fleming Nominal One-Sided *p*-values Required for Early Success at each Interim Analysis

Number of Patients Complete	Nominal One-Sided *p*-value
700	0.0015
850	0.0036
1000	0.0067
1400	0.0224

This trial will be considered a success at one of these analyses if the one-sided *p*-value for the treatment group term in the primary analysis model is less than the required nominal one-sided *p*-value shown in Table 1.

3.2 Early Futility

The predictive probability of success at the trial's maximum sample size of 1,400 patients will be used to determine if the trial should stop early for futility. This predictive probability calculation combines the knowledge of the treatment effect observed in the trial with the uncertainty of the future data we have yet to see. The predictive probability of trial success at the maximum sample size (PP_{max}) can be calculated as described in Broglio et al (2014). Let Y_{id} be the primary outcome of 28-day all-cause mortality for patient i on arm d, where $d = c$ is the placebo control arm and $d = e$ is the experimental recAP arm. We model the outcomes as

$$Y_{id} \sim \text{Bernoulli}(P_d)$$

where P_d is the underlying 28-day all-cause mortality rate on arm d. We transform the response rates onto the log-odds scale and model:

$$\theta_d = \log\left(\frac{P_d}{1 - P_d}\right).$$

The mean response is modeled independently with the same prior distribution for both arms:

$$\theta_d \sim N(0, 1.82^2).$$

The Bayesian model of the primary endpoint described above is fitted to the data at each interim analysis, and the posterior distribution of θ_d for each arm is estimated separately using only data from that arm. The posterior is calculated as:

$$p(\varphi|Y) \propto \prod_{i=1}^{n} p\,(y_i|\varphi)p(\varphi)$$

where φ is the set of parameters for the final endpoint model, $p(\varphi)$ is the prior for those parameters, y_i is the primary endpoint value for each patient, and n is the number of patients. The posterior is evaluated using MCMC with individual parameters updated by Metropolis Hastings (or Gibbs sampling where possible), using only the y_i data available at the time of the update.

To calculate the predictive probability of trial success at the maximum sample size, we sample a single mean response, θ_d, from the posterior distribution and obtain the corresponding P_d. For each patient i still in follow-up or yet to be enrolled, we sample a dichotomous response, Y_{id}, for the patient's 28-day all-cause mortality endpoint.

We conduct the trial's final analysis on the simulated complete dataset, and record trial success or failure by comparing the *p*-value obtained with the simulated complete dataset to the final required *p*-value of 0.0224. The predictive probability of trial success is the proportion of draws from the posterior distribution that result in trial success.

At the first interim analysis (N = 400 patients), this trial will stop early for futility if PP_{max} is less than 15%. At all subsequent interim analyses, it will stop early for futility if PP_{max} falls below 5%. Futility in this trial can be considered non-binding as the success boundary is calculated assuming no futility stopping.

4.0 Example Trials

In this section we describe individual simulated trials in order to illustrate the interim analyses and early-stopping decisions.

Example Trial 1

The first example trial is described in Table 2. At each interim analysis, we report the number of patients with complete information in each treatment group and the proportion that died within 28 days from randomization. We provide the current one-sided *p*-value comparing the two groups and the predictive probability of trial success at the maximum sample size.

Table 2: Example Trial 1				
Num. Patients Complete	**Control**	**Treatment**	***Current P-value***	***PP_{max}***
400	61/200 (30.5%)	58/200 (29.0%)	0.372	0.193
700	114/350 (32.6%)	100/350 (28.6%)	0.126	0.338
850	140/425 (32.9%)	129/425 (30.4%)	0.209	0.102
1000	156/500 (31.2%)	148/500 (29.6%)	0.291	0.012

The first interim analysis takes place with 400 evaluable patients, at which time 61 out of 200 patients on control (30.5%), and 58 out of 200 patients on active treatment (29.0%), died within the first 28 days. Thus, we observe an absolute difference in 28-day all-cause mortality rates of 1.5%. At this first interim, the trial may halt for futility but not success. The predictive probability of success at the maximum sample size (PP_{max}) is 19.3%, which exceeds the critical value for futility stopping for this first interim of 15%. The trial therefore continues accruing patients.

At the second interim (700 evaluable patients), 32.6% of patients on control and 28.6% of patients on treatment died in the first 28 days from randomization. The trial may now halt for either success or futility. The predictive probability of success at the maximum sample size is 33.8%, which again exceeds the critical value for futility, now at 5% for this interim analysis. The one-sided *p*-value comparing the treatment groups is 0.126, which exceeds the critical value of 0.0015 for success stopping at the second interim. Thus, the trial continues accruing patients.

The third interim analysis takes place with 850 evaluable patients. The observed rates of 28-day all-cause mortality are 32.9% on the control arm and 30.4% on the treatment arm. The results of the interim analysis resemble the previous interim: The current one-sided *p*-value is 0.209, which exceeds the critical value of 0.0036 required for early success at this interim. The predictive probability of trial success at the maximum sample size is 10.2%, which is greater than the 5% required for early futility. Therefore, the trial continues to the next interim analysis.

The next interim analysis occurs upon complete information for 1000 patients, at which time the absolute difference between rates of 28-day mortality on treatment and control is approximately 2%. The predictive probability of trial success at the maximum sample size is now 1.2%, which falls below the 5% critical value. The trial stops for futility at this interim analysis.

Example Trial 2

Table 3 summarizes the interim data available for a second individual simulated trial.

Table 3: Example Trial 2				
Num. Patients Complete	**Control**	**Treatment**	***Current P-value***	***PP_{max}***
400	80/200 (40.0%)	56/200 (28.0%)	0.006	0.953
700	142/350 (40.6%)	96/350 (27.4%)	<0.001	0.999

The first interim analysis occurs with 400 evaluable patients, at which time 40.0% of patients on the control arm and 28.0% of patients on the treatment arm died in the first 28 days from randomization. The predictive probability of success at the maximum sample size is 95.3%, which far exceeds the critical value of 15% for futility stopping. Early success stopping is not allowed at this first interim and the trial continues.

At the second interim, 142 out of 350 patients on the control arm (40.6%), and 96 out of 350 patients on the treatment arm (27.4%), died in the first 28 days of follow-up. Thus, we observe an absolute benefit in 28-day all-cause mortality of roughly 13% for the treatment arm as compared to control. The current one-sided *p*-value is less than 0.001, which falls below the critical value of 0.0015 for early success at this interim analysis. The trial stops early for success at the second interim analysis.

5.0 Operating Characteristics

In this section we characterize the average performance of the trial design.

5.1 Simulation Assumptions

We define several underlying truths for the control and treatment groups in terms of the proportion of patients on each arm with mortality in the first 28 days. We simulate 10000 trials for each of these scenarios. Drop-outs were not included in the simulations, as the number of patients who drop out or who will be lost to follow-up within 28 days is expected to be low. We simulate virtual patients in each trial and assign them a primary endpoint outcome according to a binomial distribution with probability equal to the scenario's underlying truth. Simulations are inclusive of all interim analyses as described, complete with early success and early futility decisions in place.

The primary analysis is a logistic regression model for 28-day mortality with site as a random effect and fixed effects for mSOFA, eGFR, and treatment. For the purpose of trial simulation and demonstrating the operating characteristics of the trial design, we simulate only the primary outcome of mortality, and assume the final analysis is a test of proportions between the two randomized groups. The aforementioned covariates may be associated with the primary outcome; thus, one may view the probabilities of 28-day all-cause mortality used in each simulation scenario as being the probabilities of 28-day all-cause mortality after accounting for the relationship between the covariates and the outcome in each treatment group. The uncertainty in how these covariates will impact the outcome is taken into account by simulating across a range of possible 28-day all-cause mortality rates on the control arm and a range of possible treatment effects. Hence, although the clinical trial simulation and corresponding operating characteristics presented here do not explicitly take into account the covariates and full primary-analysis model, the operating characteristics are nonetheless accurate for the trial's operating characteristics with inclusion of the covariates.

During conduct of the trial, at each interim evaluating early success as well as at the final analysis, the full primary analysis model will be used. The group sequential approach to early

success stopping remains valid when applied to the full primary-analysis model. Due to the complexity in simulating future data with the appropriate correlations between multiple covariates, the predictive probability calculation will continue to be based solely upon observed mortality proportions in each group.

5.2 Operating Characteristics

For each underlying truth, we simulate 10000 individual trials and track the behavior of each trial, including but not limited to the final outcome of the trial. The results in this section are summarized across all simulated trials for each scenario. Table 4 reports the operating characteristics for this trial design, including the total probability of trial success, the mean sample size, the probability of early success, and the probability of early futility. Early success is defined as achieving trial success at one of the interim analyses prior to the final planned analysis at 1,400 patients. Similarly, early futility is defined as declaring trial futility at one of the interim analyses prior to the final planned analysis at 1,400 patients.

We set the control 28-day all-cause mortality rate at 35% across all scenarios, which was approximately the rate of 28-day all-cause mortality observed in the previous phase II trial. Results for other control rate assumptions are shown in the appendix. We explore a range of treatment effects from a 0% to a 15% absolute improvement in the proportion of patients with 28-day all-cause mortality. The scenario in which treatment and control rates of 28-day all-cause mortality are the same (0% improvement) represents the null hypothesis. The total probability of trial success in that scenario can be interpreted as the trial's simulated Type I error.

Early success in this trial is defined by the Lan DeMets approximation to the O'Brien-Fleming spending function. As such this trial has analytical control of the overall Type I error across the entire null space. The futility boundary in this trial can be considered non-binding. However, the trial was simulated assuming that the futility recommendation would always be followed; as such, the simulated Type I error is less than the one-sided 2.5% level.

Table 4: Operating Characteristics

Control	Treatment	Absolute Improvement	Probability of Success (Power)	Mean N	Probability Early Success	Probability Early Futility
35%	35%	0%	0.022	696	0.006	0.856
35%	33%	2%	0.103	857	0.038	0.663
35%	31%	4%	0.310	1005	0.132	0.425
35%	30%	5%	0.461	1054	0.216	0.308
35%	29%	6%	0.616	1067	0.333	0.211
35%	28%	7%	0.753	1050	0.472	0.136
35%	27%	8%	0.856	1010	0.614	0.082
35%	26%	9%	0.925	960	0.741	0.046
35%	25%	10%	0.966	906	0.843	0.023
35%	20%	15%	1.000	757	0.998	0.000

In the scenario where both the control and the treatment are equal at a 35% rate of 28-day all-cause mortality, we see that the overall simulated one-sided Type I error is 0.022. In this

scenario there is an 85.6% probability of early futility and the mean sample size is 696 patients. If there is a 6% treatment benefit, this trial has 61.6% power with a 33.3% probability of early success and a mean sample size of 1067. If there is a 10% treatment benefit, this trial has 96.6% power and the mean sample size is decreased to 906 due to an increase in the probability of early success. Among all simulated trials, the smallest difference between treatment and control that was observed to achieve success at the maximum sample size of 1,400 patients was approximately 5%.

6.0 References

1. Broglio KR, Connor JT, Berry SM. Not Too Big, Not Too Small: A Goldilocks Approach To Sample Size Selection. *Journal of Biopharmaceutical Statistics*. 2014;24(3):685-705. doi:10.1080/10543406.2014.888569

2. Jennison C, Turnbull BW. *Group Sequential Methods with Applications to Clinical Trials.* Boca Raton: Chapman & Hall/CRC, 2000. Print.

7.0 Appendix

Table 5: Operating Characteristics Under 28-day Mortality Rate of 30% on Control						
Control	Treatment	Absolute Improvement	Probability of Success (Power)	Mean N	Probability Early Success	Probability Early Futility
30%	30%	0%	0.020	694	0.008	0.856
30%	26%	4%	0.340	1022	0.146	0.396
30%	25%	5%	0.507	1065	0.245	0.273
30%	24%	6%	0.665	1067	0.381	0.179
30%	23%	7%	0.796	1036	0.532	0.112
30%	22%	8%	0.891	988	0.673	0.065
30%	21%	9%	0.947	929	0.797	0.035
30%	20%	10%	0.975	873	0.887	0.019
30%	15%	15%	1.000	750	1.000	0.000

Table 6: Operating Characteristics Under 28-day Mortality Rate of 40% on Control						
Control	Treatment	Absolute Improvement	Probability of Success (Power)	Mean N	Probability Early Success	Probability Early Futility
40%	40%	0%	0.023	695	0.007	0.854
40%	36%	4%	0.291	1003	0.123	0.435
40%	35%	5%	0.430	1046	0.201	0.327
40%	34%	6%	0.582	1062	0.309	0.235
40%	33%	7%	0.718	1059	0.434	0.152
40%	32%	8%	0.828	1028	0.567	0.095
40%	31%	9%	0.902	977	0.695	0.059
40%	30%	10%	0.949	925	0.799	0.035
40%	25%	15%	0.999	764	0.994	0.001

11 References

[1] Brown SM, Lanspa MJ, Jones JP, et al., Survival After Shock Requiring High-Dose Vasopressor Therapy. Chest. 2013 Mar;143(3):664-671.

[2] Bellomo R, Ronco C, Kellum JA, et al. Acute renal failure - definition, outcome measures, animal models, fluid therapy and information technology needs: the Second International Consensus Conference of the Acute Dialysis Quality Initiative (ADQI) Group. Crit Care. 2004;8(4):R204-12.

[3] Horio M, Imai E, Yasuda Y et al. Modification of the CKD epidemiology collaboration (CKD-EPI) equation for Japanese: accuracy and use for population estimates. Am J Kidney Dis 2010; 56: 32–38.

[4] Fleischmann C, Scherag A, Adhikari NK, et al; International Forum of Acute Care Trialists. Assessment of global incidence and mortality of hospital-treated sepsis; Current estimates and limitations. Am J Respir Crit Care Med. 2016;193(3):259-272.

[5] Singer M, Deutschman CS, Seymour CW, et al. The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). JAMA. 2016;315(8): 801-810.

[6] Kaukonen KM, Bailey M, Suzuki S, Pilcher D, Bellomo R. Mortality related to severe sepsis and septic shock among critically ill patients in Australia and New Zealand, 2000-2012. JAMA. 2014;311(13):1308-1316.

[7] Iwashyna TJ, Cooke CR, Wunsch H, Kahn JM. Population burden of long-term survivorship after severe sepsis in older Americans. J Am Geriatr Soc. 2012;60(6):1070-1077. doi: 10.1111/j.1532-5415.2012.03989. x. Epub 2012 May 29.

[8] Stevenson EK, Rubenstein AR, Radin GT, Wiener RS, Walkey AJ. Two decades of mortality trends among patients with severe sepsis: a comparison meta-analysis. Crit Care Med. 2014;42(3):625-631.

[9] Vincent JL, Lefrant JY, Kotfis K, et al; ICON and SOAP investigators; SOAP investigators. Comparison of European ICU patients in 2012 (ICON) versus 2002 (SOAP). Intensive Care Med. 2018;44(3)337-344. doi:10.1007/s00134-017-5043-2.

[10] Rhee C, Dantes R, Epstein L, et al. Incidence and trends of sepsis in US hospitals using clinical vs claims data, 2009-2014. JAMA. 2017;318(13):1241-1249.

[11] Richter DC, Heininger A, Brenner T, et al. Bacterial sepsis: diagnostics and calculated antibiotic therapy. Anaesthesist. 2017;66(10):737-761. doi: 10.1007/s00101-017-0363-8.

[12] Vincent JL, Rello J, Marshall J, et al. International study of the prevalence and outcomes of infection in intensive care units. JAMA. (2009;302(21):2323-2329.

[13] Angus DC, Linde-Zwirble WT, Lidicker J, Clermont G, Carcillo J, Pinsky MR. Epidemiology of severe sepsis in the United States: analysis of incidence, outcome, and associated costs of care. Crit Care Med. 2001;29(7):1303-1310.

[14] Vincent JL, Sakr Y, Sprung CL, et al; Sepsis Occurrence in Acutely Ill Patients Investigators. Sepsis in European intensive care units: results of the SOAP study. Crit Care Med. 2006;34(2):344-353.

[15] Kellum JA, Prowle JR. Paradigms of acute kidney injury in the intensive care setting. Nat Rev Nephrol. 2018;14(4):217-230.

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

[17] Chawla LS, Amdur RL, Amodeo S, Kimmel PL, Palant CE. The severity of acute kidney injury predicts progression to chronic kidney disease. Kidney Int. 2011;79 (12):1361-1369.

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

[19] Bonventre JV, Yang L. Cellular pathophysiology of ischemic acute kidney injury. J Clin Invest. 2011;121(11):4210-4221.

[20] Wen X, Peng Z, Kellum JA. Pathogenesis of acute kidney injury: effects of remote tissue damage on the kidney. Contrib Nephrol. 2011; 174:129-137.

[21] Gomez H, Ince C, De Backer D, et al. A unified theory of sepsis -induced acute kidney injury: inflammation, microcirculatory dysfunction, bioenergetics and the tubular cell adaptation to injury. Shock. 2014;41(1):3-11.

[22] Verma SK, Molitoris BA. Renal endothelial injury and microvascular dysfunction in acute kidney injury. Semin Nephrol. 2015;35(1):96-107.

[23] Cohen J. The immunopathogenesis of sepsis. Nature. 2002;420(6971):885-891.

[24] Bagshaw SM, Lapinsky S, Dial S, et al. Acute kidney injury in septic shock: clinical outcomes and impact of duration of hypotension prior to initiation of antimicrobial therapy. Intensive Care Med. 2009;35(5):871-881.

[25] Bentala H, Verweij WR, Huizinga-Van der Vlag A, van Loenen-Weemaes AM, Meijer DK, Poelstra K. Removal of phosphate from lipid A as a strategy to detoxify lipopolysaccharide. Shock. 2002;18(6):561-566.

[26] Koyama I, Matsunaga T, Harada T, Hokari S, Komoda T. Alkaline phosphatases reduce toxicity of lipopolysaccharides in vivo and in vitro through dephosphorylation. Clin Biochem. 2002;35(6):455-461.

[27] Picher M, Burch LH, Hirsh AJ, Spychala J, Boucher RC. Ecto 5'-nucleotidase and nonspecific alkaline phosphatase. Two AMP hydrolyzing ectoenzymes with distinct roles in human airways. J Biol Chem. 2003;278(15):13468-13479.

[28] Kapojos JJ, Poelstra K, Borghuis T, et al. Induction of glomerular alkaline phosphatase after challenge with lipopolysaccharide. Int J Exp Pathol. 2003;84(3):135-144.

[29] Khundmiri SJ, Asghar M, Khan F, Salim S, Yusufi AN. Effect of reversible and irreversible ischemia on marker enzymes of BBM from renal cortical PT subpopulations. Am J Physiol. 1997;273(6 Pt 2): F849-56.

[30] Wy CA, Goto M, Young RI, Myers TF, Muraskas J. Prophylactic treatment of endotoxic shock with monophosphoryl lipid A in newborn rats. Biol Neonate. 2000;77(3):191-195.

[31] Beumer C, Wulferink M, Raaben W, Fiechter D, Brands R, Seinen W. Calf intestinal alkaline phosphatase, a novel therapeutic drug for lipopolysaccharide (LPS)-mediated diseases, attenuates LPS toxicity in mice and piglets. J Pharmacol Exp Ther. 2003;307(2):737-744.

[32] Su F, Brands R, Wang Z, et al. Beneficial effects of alkaline phosphatase in septic shock. Crit Care Med. 2006;34(8):2182-2187.

[33] Verweij WR, Bentala H, Huizinga-van der Vlag A, et al. Protection against an Escherichia coli-induced sepsis by alkaline phosphatase in mice. Shock. 2004;22(2):174-179.

[34] Marshall JC, Foster D, Vincent JL, et al. Diagnostic and prognostic implications of endotoxemia in critical illness: results of the MEDIC study. J Infect Dis. 2004;190(3):527-534.

[35] Park BS, Song DH, Kim HM, et al. The structural basis of lipopolysaccharide recognition by the TLR4-MD-2 complex. Nature. 2009;458(7242):1191-1195.

[36] Kiffer-Moreira T, Sheen CR, Gasque KC, et al. Catalytic signature of a heat-stable, chimeric human alkaline phosphatase with therapeutic potential. PLoS One. 2014;9(2): e89374.

[37] Day YJ, Huang L, Ye H, Li L, Linden J, Okusa MD. Renal ischemia-reperfusion injury and adenosine 2A receptor-mediated tissue protection: the role of CD4+ T cells and IFN-gamma. J Immunol. 2006;176(5):3108-3114.

[38] Peters E, van Elsas A, Heemskerk S, et al. Alkaline phosphatase as a treatment of sepsis-associated acute kidney injury. J Pharmacol Exp Ther. 2013;344(1):2-7.

[39] Peters E., Heemskerk S., Masereeuw R., Pickkers P. Alkaline phosphatase: a possible treatment for sepsis-associated acute kidney injury in critically ill patients. Am. J. Kidney Dis. 2014;63(3):1038-1048.

[40] Peters E, Geraci S, Heemskerk S, et al. Alkaline phosphatase protects against renal inflammation through dephosphorylation of lipopolysaccharide and adenosine triphosphate. Br J Pharmacol. 2015;172(20):4932-4945.

[41] Peters E, Heuberger JAAC, Tiessen R, et al. Pharmacokinetic Modeling and Dose Selection in a Randomized, Double-Blind, Placebo-Controlled Trial of a Human Recombinant Alkaline Phosphatase in Healthy Volunteers. Clin Pharmacokinet. 2016;55(10):1227-1237. doi: 10.1007/s40262-016-0399-y.

[42] Peters E, Mehta RL, Murray PT, et al. Study protocol for a multicentre randomised controlled trial: Safety, Tolerability, efficacy and quality of life Of a human recombinant alkaline Phosphatase in patients with sepsis-associated Acute Kidney Injury (STOP-AKI). BMJ Open. 2016;6(9): e012371. doi:10.1136/bmjopen-2016-012371.

[43] Pickkers P, Mehta RL, Murray PT, et al; STOP-AKI Investigators. Effect of Human Recombinant Alkaline Phosphatase on 7 Day creatinine clearance in patients with sepsis-associated acute kidney injury: a randomized clinical trial. JAMA. 2018;320(19):1998-2009. doi:10.1001/jama.2018.14283.

[44] Kidney Disease: Improving Global Outcomes (KDIGO) Acute Kidney Injury Work Group. KDIGO Clinical Practice Guideline for Acute Kidney Injury. Kidney Int Suppl. 2012; 2: 1-138.

KDIGO 2012 Clinical practice Guidelines for the evaluation and management of chronic kidney disease. Kidney Int. 2013(Suppl 3). http://www.kdigo.org/clinical_practice_guidelines/pdf/CKD/KDIGO_2012_CKD_GL.pdf.

[45] Bjørn M, Brendstrup C, Karlsen S, Carlsen JE. Consecutive screening and enrollment in clinical trials: the way to representative patient samples? J Card Fail. 1998;4(3):225-230.

[46] Levey AS, Stevens LA, Schmid CH, et al; CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration). A new equation to estimate glomerular filtration rate. Ann Intern Med. 2009;150(9):604-612.

[47] Levey AS, Coresh J, Greene T, et al; CKD-EPI. Using standardized serum creatinine values in the modification of diet in renal disease study equation for estimating glomerular filtration rate. Ann Intern Med. 2006;145(4):247-254.

[48] Vincent JL, Moreno R, Takala J, et al. The SOFA (Sepsis-related Organ Failure Assessment) score to describe organ dysfunction/failure. On behalf of the Working Group on Sepsis-Related Problems of the European Society of Intensive Care Medicine. Intensive Care Med. 1996;22(7):707-710. PMID 8844239.

[49] Vincent JL, de Mendonça A, Cantraine F, et al. Use of the SOFA score to assess the incidence of organ dysfunction/failure in intensive care units: results of a multicenter, prospective study. Working group on "sepsis-related problems" of the European Society of Intensive Care Medicine. Crit Care Med. 1998;26(11):1793-1800. PMID 9824069.

[50] Bellomo R., Kellum JA, Ronco C. Acute kidney injury. Lancet. 2012;380(9843):756-766.

[51] Brookmeyer R, Crowley J.A Confidence Interval for the Median Survival Time. Biometrics. 1982:38:29–41.

Investigator Agreement Page

Declaration of the Principal or Global Coordinating Investigator

Protocol Title: A Randomized, Double-Blind, Placebo-Controlled, Two-Arm Parallel-Group, Multi-Center Phase 3 Pivotal Trial to Investigate the Efficacy and Safety of Recombinant Human Alkaline Phosphatase for Treatment of Patients with Sepsis-Associated Acute Kidney Injury

This trial protocol was subjected to critical review and has been approved by the Sponsor. The information it contains is consistent with the current risk/benefit evaluation of the investigational product as well as with the moral, ethical, and scientific principles governing clinical research as set out in the Declaration of Helsinki and the guidelines on Good Clinical Practice.

Signature

June 8th 2022
Date

P. PICKKERS
Name (block letters)

PROF. DR.
Title (block letters)

RADBOUDUMC NIJMEGEN
Institution (block letters)

+31-6-52592982
Phone number

Declaration of the National Coordinating Investigator

Protocol Title: A Randomized, Double-Blind, Placebo-Controlled, Two-Arm Parallel-Group, Multi-Center Phase 3 Pivotal Trial to Investigate the Efficacy and Safety of Recombinant Human Alkaline Phosphatase for Treatment of Patients with Sepsis-Associated Acute Kidney Injury

This trial protocol was subjected to critical review and has been approved by the Sponsor. The information it contains is consistent with the current risk/benefit evaluation of the investigational product as well as with the moral, ethical, and scientific principles governing clinical research as set out in the Declaration of Helsinki and the guidelines on Good Clinical Practice.

National Coordinating Investigator

_____ _____
Signature Date

_____
Name (block letters)

_____
Title (block letters)

_____
Institution (block letters)

_____
Phone number

Declaration of the Investigator

Protocol Title: A Randomized, Double-Blind, Placebo-Controlled, Two-Arm Parallel-Group, Multi-Center Phase 3 Pivotal Trial to Investigate the Efficacy and Safety of Recombinant Human Alkaline Phosphatase for Treatment of Patients with Sepsis-Associated Acute Kidney Injury

All documentation for this trial that is supplied to me and that has not been previously published will be kept in the strictest confidence. This documentation includes this trial protocol, Investigator Brochure, (IB), /electronic data capture (EDC) system/electronic CRF (eCRF), and other scientific data.

The trial will not be commenced without the prior written approval of a properly constituted Institutional Review Board (IRB) or Independent Ethics Committee (IEC). No changes will be made to the trial protocol without the prior written approval of the Sponsor and the IRB or IEC, except where necessary to eliminate an immediate hazard to the patients.

I have read and understood and agree to abide by all the conditions and instructions contained in this protocol.

Responsible Investigator of the local trial center

_____ _____

Signature Date

_____

Name (block letters)

_____

Title (block letters)

_____

Institution (block letters)

_____

Phone number