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Study protocol of a randomised, double-blind, placebo-controlled, two-arm parallel-group, multi-centre 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

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ABSTRACT

Introduction Sepsis, the leading cause of acute kidney injury (AKI), is associated with a high morbidity and mortality. Alkaline phosphatase (ALP) is an endogenous detoxifying enzyme. A recombinant human ALP compound, ilofotase alfa, showed no safety or tolerability concerns in a phase 2 trial. Renal function improvement over 28 days was significantly greater in the ilofotase alfa group. Moreover, a significant relative reduction in 28-day all-cause mortality of >40% was observed. A follow-up trial has been designed to confirm these findings.

Methods and analysis This is a phase 3, global, multi-centre, randomised, double-blind, placebo-controlled, sequential design trial in which patients are randomly assigned to either placebo or 1.6 mg/kg ilofotase alfa. Randomisation is stratified by baseline modified Sequential Organ Failure Assessment (mSOFA) score and trial site. The primary objective is to confirm the survival benefit with ilofotase alfa by demonstrating a reduction in 28-day all-cause mortality in patients with sepsis-associated AKI requiring vasopressors. A maximum of 1400 patients will be enrolled at ~120 sites in Europe, North America, Japan, Australia and New Zealand. Up to four interim analyses will take place. Based on predefined decision rules, the trial may be stopped early for futility or for effectiveness. In addition, patients with COVID-19 disease and patients with 'moderate to severe' chronic kidney disease are analysed as 2 separate cohorts of 100 patients each. An independent Data Monitoring Committee evaluates safety data at prespecified intervals throughout the trial.

Ethics and dissemination The trial is approved by relevant institutional review boards/independent ethics committees and is conducted in accordance with the ethical principles of the Declaration of Helsinki, guidelines of Good Clinical Practice, Code of Federal Regulations and all other applicable regulations. Results of this study will determine the potential of ilofotase alfa to reduce mortality in critically ill patients with sepsis-associated AKI and will be published in a peer-reviewed scientific journal.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ Apart from the primary endpoint, results of this trial may also illustrate the efficacy of ilofotase alfa on renal endpoints and other organ-specific clinical outcomes.
⇒ The trial was designed with input from or review by the US Food and Drug Administration, the European Medicines Agency, Japanese Pharmaceuticals and Medical Devices Agency and several national medicinal regulatory authorities in Europe, Australia and New Zealand.
⇒ The group sequential trial design allows for the results to be reported in case the trial is terminated prematurely for futility.
⇒ In the additional separate COVID-19 and 'moderate to severe' chronic kidney disease cohorts, therapeutic efficacy on clinical endpoints is likely underpowered to reach statistical significance.

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INTRODUCTION

Sepsis, defined as a dysregulated host response to infection causing organ dysfunction, is the leading cause of death worldwide.[1 2] Sepsis is also the leading cause of acute kidney injury (AKI).[3–5] Patients with sepsis who develop AKI have a higher mortality, while an improved outcome is observed in patients with resolving AKI.[6 7] Patients who survive a sepsis-associated AKI episode are at risk of developing chronic kidney disease (CKD), resulting in a high burden for both the patient and society.[8–10] AKI is a multifactorial condition with inflammatory, direct nephrotoxic and ischaemic insults combining with other pathogenic responses to rapidly cause dysfunction or failure of the kidney.[11–14] Currently, there are no pharmacological interventions approved for the treatment of sepsis-associated AKI, and renal replacement therapy is the only supportive treatment option available for these patients.[15]

Alkaline phosphatase (ALP) is an endogenous homodimeric enzyme present in many cells and organs (eg, intestines, placenta, liver, bone, kidney and granulocytes) with detoxifying effects through dephosphorylation of endotoxins[16 17] and other pro-inflammatory compounds such as ATP.[18 19] Local ALP concentrations reflect the host defence against endotoxin in the kidney.[20] During ischaemia, ALP levels are markedly depleted, which is associated with the development of AKI.[21] Apart from local effects in the kidney, ALP may attenuate the innate immune response, as dephosphorylation of endotoxin abolishes its biological activity and the dephosphorylated endotoxin acts as a toll-like receptor 4 antagonist.[22] In animal models of sepsis, ALP administration attenuates the inflammatory response and reduces mortality.[23 24] There is increasing evidence that ALP plays a significant role in host defence and innate immunity, particularly against inflammatory reactions due to endotoxin release.[25]

Recombinant human ALP (recAP and ilofotase alfa)

Ilofotase alfa is a full-length human chimeric ALP produced by recombinant technology. It is encoded by a human intestinal ALP sequence (highest biological activity)[26] wherein the sequence encoding the crown domain has been substituted with the corresponding human placental ALP sequence (longest half-life).[26] Ilofotase alfa has a mass of approximately 105 kDa based on the amino acid sequence derived from the DNA sequence and approximately 130 kDa as a fully glycosylated molecule.

A large phase 2a/2b proof-of-concept and dose-finding trial ('STOP-AKI', n=301) was conducted with ilofotase alfa in SA-AKI after two small phase 2 trials[27 28] with bovine ALP demonstrated attenuation of excretion of tubular injury markers and improvement in renal function. While the improvement in endogenous creatinine clearance over the first week in the STOP-AKI trial was not significantly greater in the treatment group compared with placebo, treatment with ilofotase alfa was associated with a more pronounced long-term improvement of kidney function and lower 28-day mortality.[29]

Trial objectives

The significant effect on 28-day mortality observed (as an exploratory endpoint) in STOP-AKI requires confirmation in a larger adequately powered, placebo-controlled, multi-centre trial. Therefore, the primary objective of REVIVAL (Recombinant human alkaline phosphatase SA-AKI survival trial) is to confirm or refute the study's primary endpoint, a reduction in 28-day all-cause mortality, in patients with sepsis-associated AKI treated with 1.6 mg/kg ilofotase alfa.

METHODS AND ANALYSIS

Patients

The target patient population is adult patients in the Intensive Care Unit (ICU) or intermediate care unit with sepsis requiring vasopressor support and recent onset AKI. Vasopressor support is defined as need for ≥0.1 µg/kg/min norepinephrine or equivalent for sepsis-induced hypotension for at least 1 hour despite adequate fluid resuscitation according to clinical judgement. AKI is defined by the KDIGO criteria.[30] If the patients are receiving vasopressor support and develops AKI, the study drug infusion must start within 24 hours following the AKI diagnosis. If the patient presents with AKI, the time window is 48 hours.

There is no upper age limit for this trial. Consecutive patients will be systematically screened for AKI as soon as possible following the initiation of vasopressor treatment. Informed consent will be sought in all eligible patients with sepsis-associated AKI. Inclusion criteria are described in box 1, with the full eligibility criteria in online supplemental file 1. The first patient was enrolled on 2 November 2020. If the trial continues to recruit to target sample size, it is anticipated to run for 3 years.

Trial oversight

A Trial Steering Committee (TSC) has been established by the Sponsor AM-Pharma to facilitate design of the trial, provide leadership and oversight of trial conduct in a blinded fashion, and make recommendations to the sponsor. Likewise, a Data Monitoring Committee (DMC), consisting of independent critical care and nephrology experts not otherwise involved in the trial and a statistician, has been established to 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. The DMC will also review the interim analyses reports for efficacy and notify the sponsor and the TSC in case a futility or success threshold is reached. The DMC and TSC both follow agreed charters.

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Box 1 Short list of inclusion criteria

Inclusion criteria

1. 18 years or older.
2. In the ICU or intermediate care unit for clinical reasons.
3. Have sepsis requiring vasopressor therapy, that is:
   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 1 hour despite adequate fluid resuscitation according to clinical judgement.
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, which is known or presumed to have occurred within prior 7 days days.
   or
   c. A decrease in urinary output to<0.5 mL/kg/hour for a minimum of 6 hours 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 available from the past 12 months: a CR value ≥1.5 times the age/gender/race adjusted normal value (presented in online supplemental file 1), with the increase in CR presumed to have occurred within prior 7 days days.
5. Provision of signed and dated informed consent form in accordance with local regulations.

Patient and public involvement statement

Reflecting the acute and unpredictable nature of sepsis-associated AKI there are no specific patient advocacy groups at present; closest are organisations that represent kidney diseases or sepsis in general and, although they are aware of our programme, they were not specifically consulted on the design of this study.

Trial design

This is a phase 3, multi-centre, randomised, 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 ilofotase alfa or matching placebo. The intended ilofotase alfa dose is 1.6 mg/kg (1000 U) of patient body weight up to 120 kg (a fixed dose of 192 mg >120 kg) administered as a 1-hour infusion once daily for 3 consecutive days. The first infusion is to start as soon as feasible after randomisation.

There will be three distinct SA-AKI trial populations:

1. *The main trial population*: patients with sepsis-associated AKI and a pre-AKI reference estimated glomerular filtration rate (eGFR) ≥45 mL/min/1.73 m^2 and no proven or suspected COVID-19 at time of randomisation.
2. *A 'moderate to severe' CKD population*: patients with sepsis-associated AKI a pre-AKI reference eGFR ≥25 mL/min/1.73 m^2 and <45 mL/min/1.73 m^2 and no proven or suspected COVID-19 at time of randomisation.
3. *A COVID-19 population*: patients with proven or suspected COVID-19 at time of randomisation with or without 'moderate to severe' CKD.

Participating sites that formally stated that they would commit to follow the internationally accepted standard of care guidelines for sepsis,[31] and those for AKI[30 32] were selected. If new versions of these guidelines are published during the trial, these will be adopted.

A minimum of approximately 450 and a maximum of 1400 patients in the main trial population are planned to be enrolled at approximately 120 sites predominantly across Europe, North America, Japan, Australia and New Zealand. Additionally, approximately 100 patients in the 'moderate to severe' CKD population and up to 100 patients in the COVID-19 population are planned to be enrolled to generate exploratory data on safety and effects of ilofotase alfa in these populations. The final number of patients enrolled will depend on the recommendations of the DMC based on the safety data reviews and interim analyses applying predefined decision rules to determine futility/success.

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 1000 evaluable patients (ie, treated patients in the main trial population who have reached day 28). At the first interim analysis, the trial may be stopped for futility only. At subsequent interim analyses, the trial may be stopped for futility, or success (ie, early demonstration of superiority of ilofotase alfa over placebo on 28-day all-cause mortality). More details of the safety data reviews and the interim analyses are provided in the DMC charter and Statistical Analysis Plan (SAP), which were finalised before the first safety data review.

The trial includes a pre-treatment period, during which the patient is screened, baseline assessments are performed and the patient is randomised, a treatment period from day 1 to day 3, during which the patient receives a daily 1 hour continuous intravenous infusion of 50 mL trial drug (ilofotase alfa 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 are performed. The trial drug is provided on top of standard of care specified by the Surviving Sepsis Campaign guidelines and KDIGO guidelines. Safety is followed up until study day 28 (inclusive). All serious adverse events that occur after study 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. All deaths will be recorded up to study day 180. The trial design is presented in figure 1, the timelines for eligibility in figure 2, the trial flow for the individual patient in online supplemental figure 1 and the schedule of activities in online supplemental file 2.

Statistical considerations

The primary efficacy endpoint is '28-day all-cause mortality', defined as the probability to die (from any

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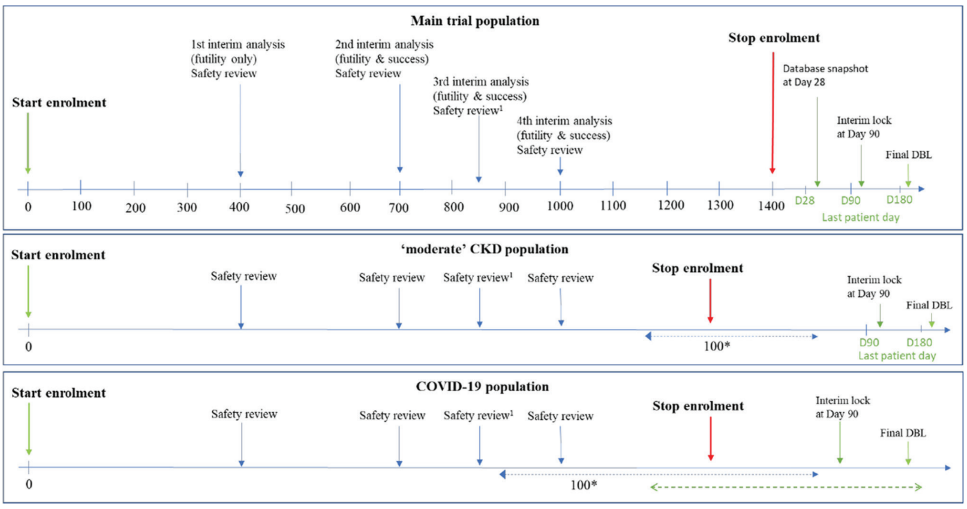

Figure 1 Trial design. Up to 1400 patients in the main trial population, up to approximately 100 patients in the 'moderate to severe' CKD population and up to approximately 100 patients in the COVID-19 population will be enrolled. There will be a maximum of four interim analyses, after approximately 400, 700, 850 and 1000 evaluable patients (ie, 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 (ie, 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 to severe' 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 1400 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 analysed and the results used to start the preparation of the CTR. The final DBL will take place after all patients have completed the trial (ie, all patients have completed day 180 or have withdrawn/are lost to follow-up prior to day 180). If patients in the 'moderate to severe' 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 to severe' 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. 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.

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.

The primary, secondary and tertiary/explorative endpoints are listed in table 1. Summary of statistical analyses and definitions of endpoints are described in online supplemental file 3.

Statistical analyses will be performed by a sponsor-designated statistician, blinded to treatment allocation. Further details of the statistical analyses are described in the SAP, which has been finalised prior to the first safety review of the trial database (online supplemental file 3). A separate DMC SAP describes the analyses to be performed for the safety data reviews and interim analyses. All statistical analyses of efficacy endpoints will be performed on the modified intention-to-treat analysis sets, defined as all patients in the population who are randomly assigned to trial drug and for whom administration of trial drug was started. All statistical analyses of safety endpoints will be performed on the safety analysis sets. Of note, after acceptance of the manuscript, an amended SAP was written and approved prior to the first interim-analysis. This amendmentment, which were not subjected to peer-review, is depicted in online supplemental file 4.

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

The Lan-DeMets approximation of the O'Brien-Fleming alpha spending function was used to determine the critical values for declaring success at interim and final analyses. As safety is evaluated by the DMC on a regular basis throughout the trial and the risk of increased mortality, that is, inferiority of ilofotase alfa compared with placebo, is controlled by the futility analyses, the test for therapeutic efficacy is a superiority test using a one-sided significance level of 0.025. Online supplemental file 3 shows

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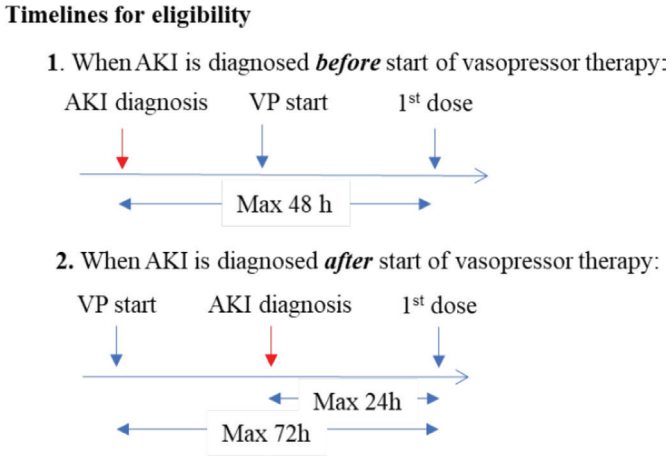

Figure 2 Timelines for eligibility. To be eligible for the trial, patients must have both sepsis requiring vasopressor therapy and AKI. (1) When AKI is diagnosed before the start of vasopressor therapy, infusion of first dose of trial drug must start within 48 hours of AKI diagnosis. (2) When AKI is diagnosed after start of vasopressor therapy, infusion of first dose of trial drug must start within 24 hours of AKI diagnosis and no more than 72 hours 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–4d. 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 1 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 12 hours without any vasopressor is needed to consider start of vasopressor therapy as a new episode. AKI,acute kidney injury; recAP, recombinant human alkaline phosphatase; VP, vasopressor.

the nominal one-sided significance levels at interim and final analyses for declaring success if data from approximately 700, 850, 1000 and 1400 patients in the main trial population will be available. This trial will be considered a success, and the intervention effective, if the one-sided p value from the primary efficacy analysis model for the treatment term is lower than the respective nominal one-sided significance level. Should the trial be stopped for early success at an interim analysis, then the analysis of the secondary endpoints within the sequential testing will proceed using the nominal one-sided significance level as allocated to the primary endpoint at the time of the analysis. The predictive probability of success at the main trial population's maximum sample size of 1400 patients will be used to determine if the trial should stop early for futility. Futility stopping recommendation in this trial is considered to be non-binding.

For this trial design, the operating characteristics (type I error probability and power) are derived via simulations for a maximum sample size of 1400 patients in the main trial population, and the group sequential design. Twenty-eight 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 subgroup of patients with a study baseline eGFR <60 mL/min in STOP-AKI. In case of an 8% absolute survival benefit of the treatment, this trial has 85.6% power and the mean sample size is expected to be 1010 patients due to the probability of early success or futility.

Safety endpoints

Safety parameters will be evaluated on the Safety Set. Incidence of (Serious) Adverse Events and Treatment Emergent Adverse Events categorised by MedDRA System Organ Class and Preferred Term will be summarised by trial drug group. Adverse event seriousness, severity, relationship to trial drug and whether leading to discontinuation of trial drug will also be recorded. Anti-drug antibodies results will be listed, including the results of the screening test and, if needed, the results of the confirmatory test and titre determination per dose group.

Population PK

A population pharmacokinetics (PK) analysis of plasma concentration-time data will be performed using non-linear mixed-effects modelling. Data from this trial may be combined with data from the phase 1 PK, safety and tolerability trial in Caucasian healthy adult volunteers,[33] an additional PK, safety and tolerability trial performed in Japan in Japanese healthy adult volunteers, and the phase 2 trial (STOP-AKI) in patients with SA-AKI[29] 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.

Sensitivity analyses and secondary endpoints

Sensitivity analyses, based on baseline disease severity score and time from fulfilling inclusion criteria to time of treatment, will be performed on the primary endpoint. In addition, day 28 all-cause mortality obtained based on Kaplan-Meier curves for time to death up to day 28 will be compiled separately for the mSOFA categories (≤9 vs >9) and combined. Finally, a tipping point analysis in which all ilofotase alfa patients with missing data on survival status on day 28 will be considered as being dead, while all placebo patients with missing data will be considered alive, and all possible combinations of missing data between these two extremes will be considered.

The most relevant secondary endpoint is Major Adverse Kidney Events (MAKE) at day 90, defined as dead, or on renal replacement therapy at day 90, or ≥25% decline of eGFR on day 28 and day 90 relative to the pre-AKI reference level. An additional MAKE90a endpoint was added (prior to unblinding) existing of: death up to and including day 90, >25% decline of eGFR at day 90 relative to pre-AKI

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Table 1 Primary and secondary endpoints

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 MAKE	MAKE 90: 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. Receiving RRT through day 28. Rehospitalisation up to and including day 90.
To investigate the effect of recAP on use of organ support, that is, MV, RRT, vasopressors or inotropes	Days alive and free of organ support through day 28, that is, 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 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 mSOFA scores defined as the SOFA score without the 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 first)
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 rehospitalisation	Days alive and out of the hospital through day 90 (with death within 28 days counting as zero days) Incidence of at least one rehospitalisation at any hospital through day 90
To investigate the effect of recAP on QoL	Change in index values, QALY and VAS score based on the 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, cAMP, and adenosine) through day 4 at selected sites
PK	
To investigate the 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

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 to severe' CKD population and the COVID-19 population without any formal hypothesis testing.
cAMP, cyclic AMP; CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; EQ-5D-5L, EuroQoL-5-Dimention-5 Levels; GCS, Glasgow Coma Scale; ICU, Intensive Care Unit; LOS, length of stay; MAKE, major adverse kidney events; mSOFA, modified Sequential Organ Failure Assessment; MV, mechanical ventilation; PK, pharmacokinetics; QALY, quality-adjusted life years; QoL, quality of life; recAP, recombinant human alkaline phosphatase; RRT, renal replacement therapy; SAEs, serious adverse events; VAS, visual analogue scale.

reference level, receiving RRT at day 90 or receiving RRT through day 28, rehospitalisation up to and including day 90, and an exploratory analysis to evaluate the influence of pre-existent renal function on the therapeutic efficacy of ilofotase alfa. Additional secondary endpoints are: days alive and free of organ support through day 28 (with death within 28 days counting as zero days), days alive and out of the ICU through day 28 (with death within 28 days counting as zero days) and time to death through day 90.

Tertiary/explorative endpoints are: time to death through day 180, change in total and individual organ failure scores through day 7 and the effect of recAP on short-term and long-term renal function, cardiovascular function, pulmonary function, hospital length of stay and quality of life.

ETHICS AND DISSEMINATION

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 Organisations of Medical Sciences' International Ethical Guidelines; applicable International Conference on Harmonisation GCP Guidelines; and applicable laws and regulations. The protocol, substantial protocol amendments, informed consent form (ICF), investigator's brochure

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(IB) and other relevant documents (eg, any other written information regarding this trial to be provided to the patient or the patient's legal representative) will be submitted to an IRB/IEC by the investigator and reviewed and approved by the IRB/IEC prior to being used in the trial. The Standard Protocol Items Recommendations for Interventional Trials checklist is added in online supplemental file 5.

By the very nature of the trial, it is anticipated that many eligible patients will not be able to give fully informed consent themselves due to various reasons, including disease severity, sedation or unconscious state. In this situation, the patient's legal representative may provide written consent, as approved by the institutional-specific guidelines and legislation in the respective country. Informed consent may be obtained from an independent consulting physician or based on an emergency study protocol by the investigator in countries where regulation and institution guidelines permit, and the consent procedure has been approved by the institutional review board (IRB)/independent ethics committee (IEC) or national authorities, as applicable in specific countries. In cases where the initial informed consent is obtained from a legal representative, an independent consulting physician, or by the investigator, the patient is asked to give written informed consent with the most current version of the ICF(s) as soon as they are able. The master informed consent form is attached as a online supplemental file 6.

Patients will be assigned a unique patient identification number via the Interactive Response Technology (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.

Irrespective of whether the trial is completed or prematurely terminated, the sponsor will ensure that the trial results will be posted on publicly available clinical trial registries in accordance with their requirements. In addition, results will be presented at international congresses and published in peer-reviewed journals.

In addition to answering the question, whether ilofotase alfa leads to a reduction in 28-day all-cause mortality, the study will provide important information on the effect of ilofotase alfa on MAKE and other clinical outcomes, and significantly expand the safety profile of the drug. Moreover, compared with STOP-AKI where follow-up was limited to 90 days, follow-up of patients for a period of 180 days allows for assessment of potential disease-modifying effects of ilofotase alfa on kidney function and investigation of the long-term effect on mortality and other clinically relevant, patient-centred and health-economic outcomes. Finally, biomarker analysis may improve the understanding of the mechanism of action of ilofotase alfa.

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Supplemental file 1 - Eligibility criteria

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. In order to enrol 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 enrolment criteria, also known as protocol waivers or exemptions, are not permitted.

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 fulfil 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, 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 available from the past 12 months: a CR value greater or equal to the levels presented in the Table, with the increase in CR presumed to have occurred within prior 7 days.

Table: Gender and Race Corrected Cut-off Values for Serum or Plasma CR Based on 1.5 Times Estimated Normal Values for Age Group2 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 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 to severe' (eGFR 25-45 mL/min/1.73 m^2) CKD patients is allowed, patients with 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 to severe' CKD patients is NOT allowed, patients with 'moderate to 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 to 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 to severe' CKD are excluded from the main trial population. However, as CKD patients are more prone to SA-AKI, a limited number of 'moderate to severe' CKD patients with eGFR ≥25-45 mL/min/1.73 m2 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 m2, 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.

Clinical symptoms and signs following 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 lost to follow up, 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-enrolment or concurrent participation in observational, non-interventional trials using no protocolized treatments or procedures may be allowed. Co-enrolment 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 may be 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) or other devices that support hemodynamics.
These patients also have a higher 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 as a cause of 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 m2 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. 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 m2 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.

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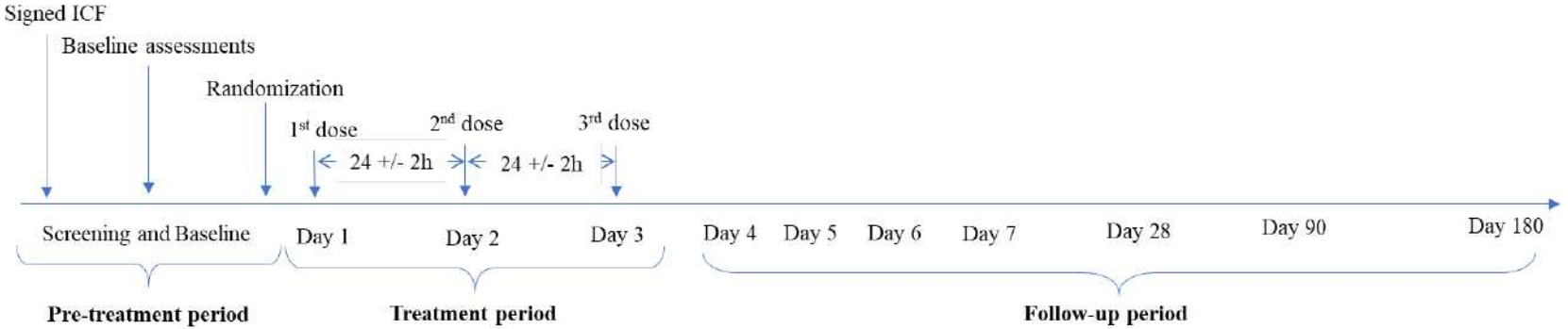

Figure 3: Trial Flow for the Individual Patient

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.

Supplemental file 2 - 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.

	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.

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. 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.
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.
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.
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. 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. 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.
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.
17 Mechanical ventilation (MV) is defined as any positive pressure ventilation via endotracheal or tracheostomy tube or any non-invasive ventilation with >5 cm H2O pressure. 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.
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.
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.
22 ICU or intermediate care unit and hospital admission and discharge dates as well as reason for admissions must be recorded.
23 Rehospitalization is defined as an overnight stay in any hospital.
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.

Supplemental file 3 – Statistical analyses and definitions of endpoints

Efficacy Analyses

Efficacy analyses will be performed and presented separately for each population. 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 to severe' CKD and COVID-19 populations.

Efficacy analyses will be performed for the mITT analysis set, which is defined as 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 analysis set will be considered as the primary analysis set for the efficacy analyses. Enrolment continues during interim analyses hence if the trial is stopped early due to futility or success, the mITT will include overrun patients.

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) from the date of randomization 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.

The treatment effect of recAP compared with placebo 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.

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 survival 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.

The primary endpoint will be analyzed using the **actual** main trial population, defined based on the pre-AKI reference eGFR (≥45 mL/min/1.73 m^2) provided in the **eCRF** in order to assess the impact of mis-allocations. In the primary analysis mentioned above in Section 7.8.1, population assignment was taken from IRT.

Subgroup analyses will be performed for the following subgroups:
• Region (US/non-US)
• Age (<65, ≥65 years)
• Gender
• mSOFA (<=9, >9)
• Other regions (North America, Europe/Australasia, Japan)
• Proven/confirmed infection type at Baseline (proven at Baseline, suspected at Baseline + confirmed later and suspected at Baseline + not confirmed later)
These analyses will use the same model as for the analysis of the primary endpoint in the mITT population, restricted to the respective subgroups. Forest plots of subgroup-specific 2-sided 95% CIs for the difference in 28-day all-cause mortality probabilities will be presented.

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 earlier; 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.
If the trial is stopped early the secondary analysis will be repeated in the interim analysis set (excluding sensitivity analyses), using the nominal 1-sided significance level allocated to the primary endpoint at the time of the interim 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.
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.

Days Alive and Free of Organ Support Through Day 28 and Days Alive and Out of the ICU Through Day 28
Days alive and free of organ support through Day 28 is defined as the number of days alive with no MV, RRT, vasopressors or inotropes, and with death within 28 days counting as zero days. Days alive and out of the ICU through Day 28 is defined as the number of days alive and out of the ICU through Day 28 with death within 28 days counting as zero days.
For both of these secondary efficacy endpoints, 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 analyses 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 analyses will be conducted for these endpoints:
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.

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 survival 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 (Brookmeyer R, Crowley JA. Confidence interval for the median survival time. Biometrics 1982; 38: 29-41).
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. 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.

Analysis of Exploratory Efficacy Endpoints
Descriptive statistics will be provided for the following exploratory efficacy endpoints by trial drug group for the mITT sets of the main trial population, 'moderate to severe' CKD population and COVID-19 population and, if applicable, a nominal 1-sided significance level of 0.025 will be used and/or 2-sided 95% CIs will be provided..

• 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 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.

Further exploratory endpoints are defined for each population, as applicable to that population. These exploratory endpoints are pre-defined (i.e., defined prior to the trial being unblinded for the first safety data review) in the trial Statistical Analysis Plan.

Analysis of Safety Endpoints
Safety parameters will be evaluated on the Safety Analysis Set, which is defined as all patients in the population who received any trial drug. Patients will be analyzed according to the first infusion of trial drug they actually received.
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.

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.

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.

Interim Analyses
A maximum of four unblinded interim analyses will be conducted.
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, recruitment may be stopped for futility. At subsequent interim analyses, recruitment 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 1 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 1: 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 ednpoints within the sequrntial 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 (PPmax) 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.

AM PHARMA

Title: Exploratory analyses following first interim analysis for the REVIVAL trial	**Document number:** SAP-CLIN-REVIVAL-001-v1.0-FINAL

Title: Exploratory analyses following first interim analysis for the REVIVAL trial

Study: AP-recAP-AKI-03-01

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

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AM PHARMA

Title: Exploratory analyses following first interim analysis for the REVIVAL trial	**Document number:** SAP-CLIN-REVIVAL-001-v1.0-FINAL

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AM PHARMA

Title: Exploratory analyses following first interim analysis for the REVIVAL trial	**Document number:** SAP-CLIN-REVIVAL-001-v1.0-FINAL

1. INTRODUCTION

Results from the first interim analysis of approximately 400 main trial population patients from the AP-recAP-AKI-03-01 (REVIVAL) trial were presented to and reviewed by the Data Monitoring Committee (DMC), as planned, during June 2022. Safety data were also presented to and reviewed by the DMC for the moderate CKD and COVID-19 populations. Following the review of the results from the first interim analysis, the recommendation from the DMC was to stop the REVIVAL trial based on futility according to the pre-defined thresholds for predictive probability of success. The DMC did not express any concerns with the safety data presented.

Blinded total group exploratory analyses were conducted by AM-Pharma and PHASTAR to determine if potential reasons why the criteria for futility had been met would emerge, but no firm conclusions could be drawn from the blinded analyses performed, mainly because treatment group allocation remained unknown. These blinded analyses are documented separately to the analyses that will be performed as part of this SAP.

This current prospective statistical analysis plan (SAP) details the exploratory analyses that will be conducted on unblinded data by AM-Pharma and PHASTAR in accordance with the objectives defined for these analyses.

Major challenge is the heterogeneity of the syndromes included (both AKI and sepsis), major aim is to sequester the various sources of variation **(in bold the most important)**;

1. For AKI
    a. **Pre-sepsis renal function**
        i. Known or assumed
        ii. CKD 1, 2, 3A, 3B, or 4
    b. Duration (time from diagnosis to treatment)
    c. Depth ergo max AKI stage (and when?)
2. For sepsis
    b. **Severity ergo SOFA/Lactate/CRP**
        i. At baseline
        ii. Recovery pattern
    b. Preexistent morbidity
    c. Medical/Surgical
    d. Causative organ
    e. Causative micro-organism
        i. Suspected
        ii. Proven
    f. APACHE (with the note that we have a time shift)
3. For disease severity: Based on APACHE/SOFA: therapeutic efficacy might be less pronounced/absent in patients that are likely to survive anyway (low severity score) or who are likely to die anyway (high severity score).

2. OBJECTIVES

The objectives for the analyses described in this SAP include:

- Identifying an effect of ilofotase alfa on short- and long-term renal function and mortality compared to controls

- Identifying sub-groups within patients with SA-AKI that have differential benefit of ilofotase alfa compared to control

3. DATA

Following further information being provided by the DMC and further discussion within AM-Pharma, the decision was taken to stop recruitment for the REVIVAL trial on 21JUL2022. Patients ongoing in the trial are to be followed up according to the protocol until 22 August 2022 where the data cut will occur. This will result in approximately 650 patients who have a chance for follow-up through day 28 and approximately 550 patients who have a chance for follow-up to 90 days. The data that will be used in these exploratory analyses will include patients from all 3 trial populations defined in the trial protocol:

- The main trial population
- The moderate CKD population
- The Corona Virus Disease 2019 (COVID-19) population

Analysis populations will be defined as follows, and analyzed in the order of:

1. One overall combined population consisting of the main trial population, the moderate CKD population and the COVID-19 population
2. The three individual trial populations
    - Main trial population
    - Moderate CKD population
    - COVID-19 population
3. One combined population consisting of the main trial population and the moderate CKD population
4. Other combinations to be defined

For the analyses by individual trial population, patients that were incorrectly randomized into the wrong trial population based on their pre-AKI reference eGFR, but then rerandomized in the correct trial population, will be analyzed in the trial population they were rerandomized to.

For the analyses described in this SAP, patients that have relevant data will be included in the analyses described; no data imputation will be performed. However, for patients that do not complete visits due to the trial being stopped, their data may be included in the mixed model repeated measures (MMRM) analysis, if appropriate, in order to include the data they have for the visits they have completed.

All patients will be analyzed according to the treatment they received regardless of what their planned treatment was.

4. UNBLINDING

An unblinded DMC support team at Labcorp was responsible for the production and presentation of the unblinded results to the DMC. The programming of the analysis datasets and outputs was initially performed and validated by the blinded programming team at Labcorp, and the validated programs passed to the unblinded team to apply the actual treatment codes and produce the unblinded results.

For the analyses described in this SAP, responsibilities for creating unblinded data are described in the following sections. Labcorp will take the responsibility for updating their programs to create the

unblinded SDTM and ADaM datasets from which PHASTAR will create additional analysis variables and datasets as required for the analyses described in this SAP.

5. SUBGROUP ANALYSES

The endpoints to be used in the subgroup analyses are given in Table 1.

Table 1: Endpoints to be used in the subgroup analyses

Priority	Variable	Definition	Responsibility
1	MAKE90a	- Death until Day 90 - > 25% drop in eGFR at Day 90 visit (compared to pre-AKI reference eGFR) - On RRT at Day 90 **OR** on RRT through Day 28 - Rehospitalization	PHASTAR
2	MAKE90b	- Death until Day 90 - > 25% drop in eGFR (compared to pre-AKI reference eGFR) at Day 28 **AND** Day 90 - On RRT at day 90	LABCORP
3	Mortality D28	All-cause Mortality up to and including Day 28	LABCORP
4	Mortality D90	All-cause Mortality up to and including Day 90	LABCORP
5	Mortality D180	All-cause Mortality up to and including Day 180	LABCORP
6	eGFR at each timepoint	Patients on RRT at each timepoint will have an imputed value of '0' for eGFR at that timepoint	LABCORP /PHASTAR
7	Relative change from baseline in eGFR at each timepoint	Patients on RRT at each timepoint will have an imputed value of '0' for eGFR at that timepoint	LABCORP /PHASTAR

For the eGFR analyses, the available value from each timepoint will be included in a MMRM with treatment group and Day as fixed effects, mSOFA score used to randomize the patient as an ordered categorical variable (i.e. obtained from IRT), site as a random effect, and baseline eGFR as the single continuous covariate. The MMRM will be applied overall and for each of the subgroups defined in Table 2.

The variables that will be categorized into subgroups are defined in Table 2. PHASTAR are responsible for programming all subgroups as defined in Table 2.

Table 2: Variables that will be categorized into subgroups and the corresponding subgroup definitions

Priority	Variable	Subgroup definitions
1	Baseline mSOFA	Define 3 subgroups based on tertiles: Baseline mSOFA <= 33.3 percentile Baseline mSOFA from 33.3 to <= 66.6 percentile Baseline mSOFA > 66.6 percentile Present actual cut-off values in dataset/output
2	Baseline eGFR	Define 3 subgroups based on tertiles: Baseline eGFR <= 33.3 percentile Baseline eGFR from 33.3 to <= 66.6 percentile Baseline eGFR > 66.6 percentile Present actual cut-off values in dataset/output
3	KDIGO AKI Stage at baseline	Define 3 subgroups as: KDIGO AKI stage at baseline was 0 or 1 KDIGO AKI stage at baseline was 2 KDIGO AKI stage at baseline was 3
4	AKI Diagnosis relative to VP start	Define 2 subgroups as: AKI diagnosis before or at the same time as VP start AKI diagnosis after VP start Compare minimum VP start date/time with AKI diagnosis date/time and define subgroups based on this
4	Pe-AKI Reference eGFR	Define 3 subgroups based on tertiles: Pe-AKI Reference eGFR <= 33.3 percentile Pe-AKI Reference eGFR from 33.3 to <= 66.6 percentile Pe-AKI Reference eGFR > 66.6 percentile Present actual cut-off values in dataset/output
4	APACHE II	Define 3 subgroups based on tertiles: APACHE II <= 33.3 percentile APACHE II from 33.3 to <= 66.6 percentile APACHE II > 66.6 percentile Present actual cut-off values in dataset/output

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Priority	Variable	Subgroup definitions
5	Improving AKI	Define 2 subgroups as: Improvement of AKI No improvement of AKI Improvement of AKI is defined as > 0.3 mg/dL decline in SRCR (SRCR on which diagnosis of AKI was made vs baseline SRCR). In case baseline SRCR is not available OR from same sample as the SRCR on which diagnosis of AKI was made, SRCR on Day 1 is used (if Day 1 not available, Day 2 is used) Subjects on RRT at baseline/Day 1/Day 2 will be regarded "No improvement of AKI"
6	Main cause of sepsis	Define 2 subgroups as: Cause of sepsis: medical Cause of sepsis: surgical or trauma
7	Baseline CRP	Define 3 subgroups based on tertiles: Baseline CRP <= 33.3 percentile Baseline CRP from 33.3 to <= 66.6 percentile Baseline CRP > 66.6 percentile Present actual cut-off values in dataset/output
8	Baseline lactate	Define 2 subgroups as: Baseline lactate <= 2 mmol/L Baseline lactate > 2 mmol/L
9	Time from AKI diagnosis to treatment start	Define 2 subgroups as: <= median time from AKI diagnosis to treatment start > median time from AKI diagnosis to treatment start Present actual cut-off values in dataset/output
10	Mechanical ventilation status at baseline	Define 2 subgroups as: On mechanical ventilation at baseline Not on mechanical ventilation at baseline Patients that started mechanical ventilation from their date of informed consent up to and including the date/time of trial drug administration will be considered on mechanical ventilation at baseline. Patients ongoing for mechanical ventilation at their date of informed consent and/or ongoing at the date/time of trial drug administration will also be considered on mechanical ventilation at baseline. All periods of mechanical ventilation will be considered regardless of modality (invasive or non-invasive)

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Priority	Variable	Subgroup definitions
11	Baseline P/F ratio	Define 3 subgroups based on tertiles: Baseline P/F ratio <= 33.3 percentile Baseline P/F ratio from 33.3 to <= 66.6 percentile Baseline P/F ratio > 66.6 percentile Present actual cut-off values in dataset/output
12	Charlson co-morbidity index	Define 3 subgroups based on tertiles: Charlson co-morbidity index <=33.3 percentile Charlson co-morbidity index 33.3 to <=66.6 percentile Charlson co-morbidity index >66.6 percentile Present actual cut-off values in dataset/output
13	Recruitment numbers at site	Define 3 subgroups as: patients from sites with <=2 patients recruited patients from sites with 3 to 14 patients recruited patients from sites >=15 patients recruited
14	Gram-bacterial infection	Define 3 subgroups as: Gram positive Gram Negative Mixed Data taken from the Sepsis Diagnosis Confirmation page, where 'If Bacterial' is recorded as Gram pos, Gram neg or Mixed

No data imputation will be performed. For all subgroup analyses, patients that could not be assigned to a specific category because of partial or completely missing information will be included in a missing category for that variable.

For the derivation of tertiles, all relevant values available for the analysis population of interest (as defined in Section 3) will be used to determine the cut-off values to be used in the subgroup definitions.

Tables displaying the number and percentage of patients by treatment group (ilofotase alfa or placebo) and total for each subgroup will be presented. For example:

	Treatment group					
	Placebo		ilofotase alfa		Total	
	N	Number (%) that died	N	Number (%) that died	N	Number (%) that died
Number (percentage) that died*	nn	nn (xx%)	nn	nn (xx%)	nn	nn (xx%)
	Number in subgroup	Number (%) that died	Number in subgroup	Number (%) that died	Number in subgroup	Number (%) that died
Subgroup 1**	nn	nn (xx%)	nn	nn (xx%)	nn	nn (xx%)
Subgroup 2**	nn	nn (xx%)	nn	nn (xx%)	nn	nn (xx%)
Subgroup 3**	nn	nn (xx%)	nn	nn (xx%)	nn	nn (xx%)
Missing**	nn	nn (xx%)	nn	nn (xx%)	nn	nn (xx%)

* Percentages are based on N

** Percentages are based on the number within each treatment group and subgroup

6. ANALYSIS OF BIOLOGICAL ENDPOINTS

The biological endpoints for further analysis are given in Table 3.

Table 3: Biological endpoints of interest for further analysis.

Priority	Variable	Method of analysis	Responsible for programming
1	mSOFA	MMRM with treatment group and Day as fixed effects, mSOFA score used to randomize the patient as an ordered categorical variable (i.e. obtained from IRT), site as a random effect, and baseline mSOFA as the single continuous covariate	PHASTAR
1	Relative change from baseline in mSOFA	MMRM with treatment group and Day as fixed effects, mSOFA score used to randomize the patient as an ordered categorical variable (i.e. obtained from IRT), site as a random effect, and baseline mSOFA as the single continuous covariate	PHASTAR
2	Relative change from baseline in CRP	Descriptive statistics (n, mean, standard deviation, minimum, median and maximum) for Day 3 and Day 28	PHASTAR
2	Absolute change from baseline in CRP	Descriptive statistics (n, mean, standard deviation, min, median and max) for Day 3 and Day 28	PHASTAR
3	Relative change from baseline in Lactate	Descriptive statistics (n, mean, standard deviation, min, median and max) for Day 3 and Day 28	PHASTAR

AM PHARMA

Title: Exploratory analyses following first interim analysis for the REVIVAL trial	**Document number:** SAP-CLIN-REVIVAL-001-v1.0-FINAL

Priority	Variable	Method of analysis	Responsible for programming
3	Absolute change from baseline in Lactate	Descriptive statistics (n, mean, standard deviation, min, median and max) for Day 3 and Day 28	PHASTAR
4	Days alive and free of organ support MV, RRT, vasopressor through Day 28	Defined as the number of days alive up to and including Day 28 with no organ support MV or RRT or vasopressors or inotropes. Death up to and including Day 28 is counted as zero days 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. Two-sided exact 95% CIs for the difference in the medians based on the Hodges-Lehmann location shift estimate will be constructed to aid interpretation. This method does not respect the randomization according to site and mSOFA score. More details are provided below.	LABCORP
4	Days alive and free of organ support MV through Day 28	Defined as the number of days alive up to and including Day 28 with no organ support MV. Death up to and including Day 28 is counted as zero days 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. Two-sided exact 95% CIs for the difference in the medians based on the Hodges-Lehmann location shift estimate will be constructed to aid interpretation. This method does not respect the randomization according to site and mSOFA score. More details are provided below.	LABCORP

Pickkers P, *et al. BMJ Open* 2023; 13:e065613. doi: 10.1136/bmjopen-2022-065613

AM PHARMA

Title: Exploratory analyses following first interim analysis for the REVIVAL trial	**Document number:** SAP-CLIN-REVIVAL-001-v1.0-FINAL

Priority	Variable	Method of analysis	Responsible for programming
4	Days alive and free of, RRT through Day 28	Defined as the number of days alive up to and including Day 28 with no RRT. Death up to and including Day 28 is counted as zero days 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. Two-sided exact 95% CIs for the difference in the medians based on the Hodges-Lehmann location shift estimate will be constructed to aid interpretation. This method does not respect the randomization according to site and mSOFA score. More details are provided below.	LABCORP
4	Days alive and free of vasopressor through Day 28	Defined as the number of days alive up to and including Day 28 with no vasopressors or inotropes. Death up to and including Day 28 is counted as zero days 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. Two-sided exact 95% CIs for the difference in the medians based on the Hodges-Lehmann location shift estimate will be constructed to aid interpretation. This method does not respect the randomization according to site and mSOFA score. More details are provided below.	LABCORP
5	Patients alive and free of AKI on Day 7/ICU discharge and at Day 28	A patient is defined as 'free of AKI' at Day 7/ICU discharge and at Day 28 if the derived AKI stage value on the KDIGO AKI staging page is equal to zero. The number and percentage of patients with this property will be calculated for each time point.	LABCORP

Pickkers P, *et al. BMJ Open* 2023; 13:e065613. doi: 10.1136/bmjopen-2022-065613

Priority	Variable	Method of analysis	Responsible for programming
6	Patients alive and free of new onset CKD or worsening of CKD on Day 90	A patient is defined as free of new onset or worsening of CKD (defined as any increase in CKD Stage) at Day 90 if neither of the two conditions mentioned in this endpoint is present. Especially, • For patients with a pre-AKI reference CKD stage (s. Section 7.4.6) of 1 or 2: 'New onset of CKD' is defined as KDIGO CKD stage of at least 3a. • For patients with a pre-AKI reference CKD of stage 3a or higher: 'Worsening of CKD' is defined as an increase of KDIGO CKD stage (including a change from stage 3a to 3b) as compared to the pre-AKI reference CKD stage. The number and percentage of patients with this property will be calculated.	LABCORP

The p-value of the re-randomization test will be calculated as follows:

- Step 1: Calculate X0 = Median days alive and free of xxx in recAP group – median days alive and free of xxx in Placebo group.
- Step 2: Within each stratum (site and mSOFA score) permute the treatment group labels. Call this a permutation resample.
- Step 3: Calculate Xi = Median days alive and free of xxx in 'permuted recAP' group – median days alive and free of xxx in 'new Placebo' group.
- Step 4: Repeat steps 2 and 3, 1000 times to create a permutation distribution.
- Step 5: The p-value is the proportion of the 1000 resamples (X1 to X1000) that are greater than or equal to X0.

7. OTHER ANALYSES

Baseline Characteristics

Descriptive statistics will be presented for the following baseline characteristics by analysis population as defined in Section 3 and treatment group, overall and by subgroup (as defined in Table 2).

- Gender (Male, Female) (n %)
- Age (years) (median [IQR])
- Weight (kg) (median [IQR])
- Height (cm) (median [IQR])
- BMI (kg/m^2) (median [IQR])
- Race (n%)
- APACHE II score (n %)

- MV status at baseline (on/off) (n %)
- AKI stage (n %)
- AKI diagnosis eGFR (mL/min/1.73m^2) (median [IQR])
- Pre-AKI reference eGFR (mL/min/1.73m^2) (median [IQR])
- Baseline mSOFA score (median [IQR])
- Time (h) from AKI diagnosis to start of treatment (median [IQR])
- KDIGO Criteria (n %)
- Baseline CRP (mg/L) (median [IQR])
- Baseline lactate (mmol/L) (median [IQR])
- P/F ratio (median [IQR])
- Main cause of sepsis (n %)
- Charlson Comorbidity Index (median [IQR])
- Dose of norepinephrine and other vasopressors and inotropes (n %)
- Time (h) from start of vasopressor therapy to start of first IV antibiotic (median [IQR])
- Suspected vs proven infection at randomization (n %)
- Known or suspected infection site (by category: pulmonary, abdominal, urinary tract, skin or soft-tissue, CNS, unknown or other) and known or suspected pathogen (by category: viral, bacterial (Gram positive, Gram negative, mixed), other or unknown) (n %)

Further descriptive analyses

- Number of patients on RRT per day (Day 1 to Day 28) by treatment group
- For patients not on RRT: for each day (Day 1 to Day 28) and treatment group, present the number of patients with a > 0.3 mg/dL decline in baseline SRCR (SRCR on which diagnosis of AKI was made vs baseline SRCR). In case baseline SRCR is not available OR from same sample as the SRCR on which diagnosis of AKI was made, SRCR on Day 1 is used (if Day 1 not available, Day 2 is used)

Section	Item	Description	Protocol page	Manuscript page
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	1	1
	2b	All items from the World Health Organization Trial Registration Data Set	1, US IND nr and EudracCT nr	3
Protocol version	3	Date and version identifier	1	-
Funding	4	Sources and types of financial, material, and other support	-	15
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1 and 2	15
	5b	Name and contact information for the trial sponsor	1 and 2	2
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	11: rational 84: trial administration 84: publication policy includes Trial Steering cie 68: statistical considerations	-
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	85: responsibilities in trial conduct 15: DMC 79: TSC and DMC	TSC and DMC: 6
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	31: trial rationale and Background 33: pre-clinical and clinical studies 40: scientific rational for trial design	5
	6b	Explanation for choice of comparators	-	7
Objectives	7	Specific objectives or hypotheses	36: par 3	6
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	38: trial design par 4	7

Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	38: last paragraph	7
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	43-47 par 5.1 and 5.2	Figure 1 and supplemental file 1
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	47-49 par 6	Supplemental file 2
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	78 informed consent 53 section 7.1 and 7.2	-
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	NA, ICU trial with dosing in the ICU	-
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	52 par 6.5.1	-
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	40-41 par 4.1,4.2 and 4.3	9
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	23-26 figure 1,2,3 27 table 3	Figure 1
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	21 sample size and power	8-9
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	54-55 par 8	-
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	54-55 par 8	-

Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	50 par 6.3.1	-
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	54-55	-
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	50-51 par 6.3.2	-
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	51 paragraph 6.3.2.1	-
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	80-82	-
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	53-54 par 7.2	-
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	80-82	-
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	69-75 par 9.2	8, supplemental file 3
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	71-75 as off par 9.2.1.1.2	8, supplemental file 3
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	Described in SAP	Missing data: 9
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where	79 par 10.1.5	6

		further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed		
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	74-75 par 9.3	7
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	66-67 par 8.3.4.4	8, 9
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	Performed by CRO, not in protocol	-
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	86 par 10.1.13.1	10
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	86 par 10.1.13.2	10
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	78 par 10.1.3	10
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	78 par 10.1.3	10
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	79 paragraph 10.1.4	10
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	78 paragraph 10.1.2	15
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	81-82 par.10.1.7	8
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	78 par 10.1.3	-
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	80 par 10.1.6	10

	31b	Authorship eligibility guidelines and any intended use of professional writers	84 par 10.1.11	-
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	-	-
Appendices				
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	See suppl file	Supplement file 5
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	-	-

I. Patient Information Sheet

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
Protocol No.: AP-recAP-AKI-03-01
Short Title: Recombinant human alkaline phosphatase SA-AKI survival trial (REVIVAL)
Name and Address of Sponsor: AM-Pharma B.V. Stadsplateau 6 3521 AZ Utrecht The Netherlands
Principal Investigator:
Institution: < *Include name, address, and phone number*>

Introduction

You are being invited to voluntarily take part in a clinical trial called REVIVAL to investigate recombinant human alkaline phosphatase (recAP) for patients with sepsis-associated acute kidney injury (SA-AKI; this term is explained below).

In this consent form the expressions “you” and “I” refers to the patient. If you are a legally authorized representative of a patient, please remember that “you” or “I” means the patient and not you as the representative except for circumstances where you act on behalf of the patient.

This document tells you about the clinical trial and includes information about the reason why the clinical trial is being done, what will happen to you if you take part in the clinical trial, and the possible benefits and risks of participating in this clinical trial. Take time to read this document carefully and feel free to talk about it with your partner, family members, family doctor or others.

Your trial doctor or a member of the clinical trial team will also talk to you in detail about the information in this document. Ask your doctor or a member of the clinical trial team to explain anything that is not clear to you.

If you choose to take part in this clinical trial, you will be asked to sign this document. You will get a signed and dated copy of this information letter and consent form.

AM-Pharma B.V. (a pharmaceutical company) is organizing and funding this clinical trial. AM-Pharma B.V. (“AM-Pharma”) will pay the clinical trial site to cover their costs of conducting this clinical trial. If applicable, your trial doctor will disclose to you any financial links or other interests that he/she may have to AM-Pharma.

1. Purpose of this trial

Sepsis is a condition that may occur when the body overreacts to an infection. Sepsis often leads to injury of one or more organs (multi-organ dysfunction); the kidney is a frequently injured organ. The term sepsis-associated acute kidney injury (SA-AKI) is used under these circumstances. SA-AKI is associated with an increased risk of dying from sepsis. Currently, there is no drug approved for the treatment of SA-AKI. Filtering the blood by a dialysis machine is the only treatment option available for patients with SA-AKI.

Some of the procedures performed for this clinical trial will be in addition to your standard of care. If you have questions about any of the procedures, you should ask the trial doctor or a member of the clinical trial team involved in this clinical trial.

Alkaline phosphatase (AP) is a compound normally present in many human tissues. AP has many functions including helping the body fight infection and reducing organ injury during sepsis.

recAP is the short name given to the clinical trial drug that AM-Pharma is investigating as a way to reduce the mortality of patients with SA-AKI and to improve the function of their kidneys. It is a recombinant (the *rec* in recAP) molecule which means that it was made artificially.

recAP is given to patients by a small tube inserted into a vein which is called an intravenous infusion. The purpose of this clinical trial is to determine if recAP is safe and effective in reducing the severity of SA-AKI. recAP is an experimental drug as it has not been approved by any Health Authority for any treatment.

At least 450 patients and up to 1,600 patients will take part in this clinical trial at a number of locations predominately across Europe and North America. This hospital (see name, address and phone number of the hospital on the first page of this document) is one of a number of hospitals taking part in this clinical trial. The main trial doctor for REVIVAL at this hospital is named as the Principal Investigator on the first page of this document. The main trial doctor will be helped by other trial team members.

Remove this language for US: This trial has been reviewed and approved by <*Specify local Health Authority if approvals are issued*> <and> given favorable opinion by <NAME OF IRB/IEC> that the trial can take place, that are responsible for making sure that the rights of people who take part clinical trials are protected. The approval by the <*Specify name of local Health Authority if approvals are issued*> <and> given favorable opinion <NAME OF IRB/IEC> should not be thought of as an encouragement for you to take part in this trial.

2. Trial Procedures

If you are eligible and choose to participate in REVIVAL, you will be randomly assigned (like the flip of a coin) to a treatment group by a computer. You will receive either recAP or placebo (inactive treatment). You will have a 50% chance of receiving recAP and a 50% chance of

receiving placebo. A 'placebo' looks like recAP but does not have any active substance in it. No matter which treatment group you are assigned to, you will still receive all standard treatments for SA-AKI as directed by your treating physician(s). No one among the people taking care of you or among the clinical trial team will know what treatment you received until after the entire clinical trial is over. This setup creates the best way to objectively measure the effect of recAP. Your trial doctor can find out the treatment if it is needed for your health.

All trial assessments done up to the time the trial drug is started is referred to as Screening or as Baseline. From the day the trial drug is started, each day is given a number beginning with Day 1. Day 1 and Baseline procedures may be performed on the same day.

The planned duration of your participation in the clinical trial will be approximately 6 months.

Screening and Baseline:
If you agree to take part in this clinical trial, you or your legal representative will need to sign this consent form. You may have some tests to check if you can start the trial drug and information about your health status before the start of the trial drug will be collected.

- Demographic information (age, gender, race, etc.) and information about whether you were living at home, at a rehabilitation site or nursing facility before you were admitted to the hospital will be collected.
- Information will be collected about your current health, vital signs, medical history, medications and treatments you have received, and your weight and height will be measured or estimated.
- If you are able, you will complete a quality of life questionnaire.
- If you are a woman able to have children, you will have a pregnancy test. If you are pregnant, you cannot participate in the trial.
- If not already done as part of standard care, blood will be collected for standard safety tests and to see how your kidneys are functioning. In addition, a blood sample will be taken to check whether you have antibodies against recAP, and for biomarker testing. Biomarkers are substances that may provide information about how recAP works, or the causes of disease and its individual course. Biomarkers may also help identify patients who may benefit from recAP, or identify patients at increased risk for side effects.
- *For applicable sites:* A urine sample will be collected for biomarkers.
- If not already done, an ECG (electrocardiogram) which records the electrical activity of the heart will be done.
- You will be randomized to receive trial drug (either recAP or placebo).

The trial drug will be administered by intravenous infusion over 1 hour on Days 1, 2, and 3. The dose will depend on your weight.

Days 1-3:
Procedures that are to be performed on Day 1 through 3:

- Information will be collected about your current health, any side effects you may have, and medications and treatments you receive.

- Blood will be collected for kidney function tests and biomarker testing. On Day 3 only, blood will be collected for safety tests and pharmacokinetic (PK) testing. PK measures the amount of recAP in your blood at different time points.
- *For applicable sites:* A urine sample will be collected to measure the levels of biomarkers.
- You will be administered the trial drug by intravenous infusion.

Days 4-7

Procedures that are to be performed on Days 4 through 7:

- Information will be collected about your current health, any side effects you may have, and medications and treatments you receive.
- Blood will be collected for kidney function tests (while you are in ICU or Intermediate care). On Days 4 and 5 only, blood will be collected for biomarker testing. On Days 4, 5 and 7 blood will be collected for PK testing.
- *For applicable sites:* On Day 4 only, a urine sample will be collected to measure the levels of biomarkers.

Days 8-27

- If you are still in the hospital, information about certain medications and treatments you receive will be collected.
- The clinical trial team will continue to monitor and collect information on any side effects you may experience.
- If you are being discharged from hospital during this period, you will have a blood sample taken for standard safety tests before leaving the hospital.

Day 28

If you have been discharged from the hospital, you will be asked to return to the hospital on Day 28 if you are able. If you are not able to return to the hospital, you may be contacted by telephone and receive a visit at home (off-site visit by trial staff or third party vendor). Procedures that are to be performed on Day 28:

- Information will be collected about any side effects you may have, medications and treatments you have received, whether you have been in the hospital since you were discharged, and whether you are living at home, are in the hospital or living in another facility.
- If you are able, you will complete a quality of life questionnaire.
- Blood will be collected for kidney function tests and to see if you have developed antibodies against recAP. An antibody is made by the immune system to protect the body from harmful substances and may sometimes be made against certain types of medications.
- If you are still in hospital on Day 28, blood will be collected for standard safety tests.

Day 90

You will be asked to return to the hospital around Day 90 if you are able. If you are not able to return to the hospital, you may be contacted by telephone and receive a visit at home (off-site visit by trial staff or third party vendor). Procedures that are to be performed on Day 90:

- Information will be collected about whether you have been in the hospital since you were discharged and whether you are living at home, are in the hospital or living in another facility.

- If you are able, you will complete a quality of life questionnaire.
- Blood will be collected for kidney function tests and to see if you have developed antibodies against recAP.

Day 180
Data to be collected at Day 180 can be collected over the telephone:
- Information about whether you are living at home, are in the hospital or living in another facility will be collected.
- If you are able, you will complete a quality of life questionnaire.

Early Termination Visit:
If you are withdrawn from the clinical trial or choose to withdraw from the clinical trial, you will be asked for a final blood sample to perform safety tests and to see if you have developed antibodies against recAP.

If you are withdrawn or choose to withdraw from the trial, or contact with you is lost, your trial doctor will contact you (your family or caregiver) or may access your medical records or publicly available records, to determine your health/survival status.
If you wish/decide you do not/no longer want to be contacted or allow access to your medical records for follow-up information, tell your trial doctor.

Off-site Visits by trial staff or third party vendor:
If you and your trial doctor agree, it is possible that you may have procedures for Days 28 and/or 90 performed at your home where a mobile research nurse will visit you. If possible, the mobile research nurse will meet with you while you are still in the hospital to discuss and plan the home visit(s).

The mobile research nurse will be from the trial site or a company called Illingworth Research Group. If you agree to off-site visit(s) performed by this company, the site research team will complete a registration form to provide your contact information to Illingworth. Your personal data collected by Illingworth personnel for the purpose of carrying out the off-site visit(s) will be treated with strict confidentiality. During the trial, your identifying information may be held on Illingworth's restricted access server and deleted at the end of the trial. Any paper copies used by the mobile research nurse that include your identifying information will be shredded after use. Illingworth will not release your information to any third parties, except of contact information to the courier vendor if applicable.

Where couriers are involved in off-site visits for the purpose of sending your samples to a laboratory, your address will be provided to a secure group for the service to take place. Based on your location, your contact information and address may be transferred outside of your country *<and European Union>*.

Human Biological Samples

While you are in this clinical trial, you will have biological samples taken. The maximum amount of blood collected specifically for the clinical trial will not be more than 100 mL. These samples are necessary to evaluate your health and the effects of recAP and may provide new information to help better understand your disease and how recAP works. Samples will be analyzed at either the institution where you are being treated or sent to a central laboratory for analysis. Samples will be discarded after results have been obtained. The below section refers to the use of your samples.

WHAT SAMPLES MAY BE USED FOR:

a. Your samples will be used for the research purposes explained in the procedures section of this form.

b. Your samples will not be sold or used directly for the production of commercial products.

c. In case of any commercial gain based on research results from your samples, AM-Pharma B.V. will have the ownership of the research results and may file patents. The research done with your samples may help develop new products, new medical tests or treatments in the future that have commercial value. There will be no financial benefit to you for any commercial findings or products as a result of your sample use. By agreeing to take part in this clinical trial, you agree to give up your rights for any commercial value resulting from your samples and data.

d. Your samples may be provided to a laboratory for testing and research use and storage purposes done for and on behalf of AM-Pharma and its third party collaborators. Some samples being collected will be sent to Covance Central Laboratory Services in *Switzerland* or *USA* but may need to be shipped to other locations which may be in other countries during the course of the clinical trial or following completion of the clinical trial.

e. All identifiable samples will be handled in a manner to maintain your confidentiality and will be labelled with a code number and kept in locked storage. Only your clinical trial team will be able to link your samples with your identity. No one outside the trial hospital working with your samples will know your identity.

f. Your samples will be stored for up to five years after the clinical trial is published.

g. Your samples may be stored for longer than these specified periods if this is required by a regulatory or government agency.

h. If additional tests are to be performed with your samples that are not associated with this trial, biomarker research related to the effects of recAP, or for better understanding your disease, we will inform you of those details. You can decide not to give consent for these additional tests using your samples. You have the right to be told about such new tests that use your samples for a new purpose not described in this document and you have the right to refuse these new tests on those samples.

Some reports from laboratory tests done for the clinical trial at central laboratories will not be put in your health/medical record and will be kept confidential to the best of our ability within the law. You will not be provided with the results of these laboratory tests.

3. Your Responsibilities for this trial

If you decide to take part in this clinical trial, it is important that you agree to:

- Follow the instructions provided by your trial doctor or the clinical trial team;
- Go to your trial visits. As soon as you know that you will not be able to go to a clinical trial visit, please contact your trial doctor or a member of the clinical trial team to schedule a new visit.
- Truthfully answer any questions from your trial doctor or the clinical trial team when asked about any changes in your health, visits to other doctors or hospital admissions, or changes in your medication, including prescribed medications, over the counter medications, herbal remedies, and vitamins.
- If you are or are planning to take part in other clinical trials, please inform your trial doctor. Do not take part in any other clinical trials without the consent of your trial doctor while you are taking part in this clinical trial.
- Tell the trial doctor if you believe you are pregnant.
- Tell your trial doctor or a member of the clinical trial team if you change your mind about taking part in the clinical trial.

4. Possible Risks

All drugs may cause side effects and discomforts. To date, a total of 219 patients and healthy volunteers have been exposed to recAP. In healthy volunteers, minor discomfort, such as temporary dizziness and pain at the infusion site where the trial drug entered the vein, were reported. In patients with your condition who received either placebo or recAP, the most commonly reported side effects were nausea, diarrhea, constipation, and some more severe symptoms that are seen in patients with sepsis. The number and severity of side effects was similar in the patients who received recAP and the patients who received placebo. There may be other side effects and discomforts that are not yet known.

Blood draws: Most of the blood tests will be taken from catheters (tubes) that are already in one of your veins so you should not experience additional discomfort. When a sample of your blood is drawn by using a needle, you may experience some temporary discomfort, bruising, swelling or, in rare circumstances, infection at the needle site. Tell the trial doctor or a member of the clinical trial if you do not feel well after having your blood drawn.

ECG: An ECG is an electrical tracing of the heart used to monitor heart function. Small wires are attached to your body using adhesive patches in several places. You may experience temporary discomfort (pulling on the skin/skin hair) during removal of the adhesive patches. After you have an ECG, you may have mild irritation, slight redness, and itching at the places on your skin where the recording patches are placed.

Questionnaires: You will be asked to complete questionnaires. Some of the questions may make you feel anxious or upset. You do not have to answer any questions that upset you. It is important that you answer all questions as completely and truthfully as possible.

Pregnancy risks

Women: The safety of recAP during pregnancy and breast-feeding has not been tested previously in either humans or in animal studies. Therefore, if you are pregnant or breast-feeding, you may not participate in this clinical trial. If you become pregnant during the clinical trial, it is important that you tell your doctor or a member of the clinical trial team so that you can discuss whether you should continue in the trial or whether it is best that you withdraw. In any case, you will be followed to determine the outcome of the pregnancy and status of you and your child.

Because alkaline phosphatase is present in the human placenta it is theoretically possible that you could develop anti-placental antibodies after receiving the trial drug. Such antibodies could potentially interfere with your ability to have a successful pregnancy in the future. While development of anti-placental antibodies have not been detected to date in human studies, the safety experience with recAP regarding this aspect is limited.

Men: As the trial drug is very similar to natural alkaline phosphatase already present in the body, it is not expected to affect your sperm or an unborn child.

Birth Control: The trial drug is not expected to affect the eggs or sperm so as soon as the trial drug is excreted from the body, there is no need for you or your partner to use birth control. As you will only receive treatment with the trial drug for three days while you are in the intensive care unit, it is expected that the trial drug will have been cleared from your body by the time you are discharged from the hospital.

5. Possible Benefits

Possible benefits from taking part in this clinical trial may include:

- Your health problem may or may not get better from taking part in this clinical trial, it is hoped that recAP may lessen the signs and symptoms of your health problem, however such benefit cannot be guaranteed. In this clinical trial you may get placebo which means you will not receive the active drug during the clinical trial.
- Taking part in this clinical trial will help doctors learn more about recAP. This may help others with your health problem in the future.

We cannot promise that you will get any benefits from this clinical trial.

6. Alternative Treatments

The only alternative treatment for SA-AKI is supportive care such as dialysis. Regardless of whether you decide to take part in this clinical trial, you will receive all standard medical care including supportive care as clinically indicated. This is also the case if the trial drug is prematurely stopped. Your trial doctor can talk with you about the risks and benefits of supportive care.

7. Costs

You will not receive payment for participating in this clinical trial, but the trial drug will be made available to you at no charge and you will not be required to pay for any trial procedures. You may be reimbursed for any reasonable travel expenses incurred as a result of taking part in this clinical trial. You will need to provide receipts to the clinical trial site for your travel expenses.

If applicable, you will not be charged, nor will you receive payment from visits performed by the mobile research nurses.

8. Compensation for Injury

Whilst the clinical trial is not expected to pose any additional risk to you over your normal clinical treatment, if you are nonetheless injured as a result of your participation in this clinical trial, treatment for the injury will be made available through [name of physician] and [institution]. AM-Pharma will pay the costs associated with the treatment of your injury which means that these costs will not need to be paid by your medical insurance. No other compensation or payment will be available from either AM-Pharma or the trial doctor in the event of any injury. You are however not waiving any legal rights by signing this form, by accepting medical care or by accepting payment for medical expenses.

<insert insurance language as applicable>

9. Data Protection

Your identity and your personal health data will be kept confidential at all times.

<Note: this is not to be included for EU member states where consent is not considered to be the appropriate legal basis for processing personal data>: Without your consent, your data or samples cannot be used. This is why you will not be able to take part in the clinical trial if you do not give your consent to use your personal data. You must give your authorization before the trial doctor can use or share your personal data with others.

During the course of the clinical trial, the trial doctor will collect personal data, including personal health data about you and samples, which will be used for the purpose of the clinical trial as described in section 1 of this form and may help develop new tests, procedures, and commercial products. This section will describe how your personal data will be collected and used and explain your rights.

<Note: this is not to be included for EU member states where consent is not considered to be the appropriate legal basis for processing personal data>: Your consent to the use of Trial Data for the purposes of the clinical trial does not have a specific expiration date. However, you may withdraw your consent at any time. If you do take away your consent, no new information or biological samples will be taken and you may also request that no new analysis on your samples be done.

If you decide to participate in the clinical trial, personal data will be collected by your trial doctor and/or a member of the clinical trial team. The Trial Data that will be collected are:

- Personal data
  - your name, address, telephone number, email address, health insurance number, national identification number.
  - your age, gender, ethnic and racial background.
  - information about your health, medical condition and medical history and medications you take.
- Biological Samples (see section 2 above): blood and urine (for applicable sites)
- Information about your treatments and response to treatments (which includes the procedures described in this form) and data resulting from analysis of biological samples.
- Information about side effects, medical history and test results while you are taking part in the clinical trial

Use of your Personal Data

Your full identity will not be revealed on any of the clinical trial documents or samples taken and kept by AM-Pharma for their studies.

The clinical trial team will send your personal data in pseudonymized form to AM-Pharma and to representatives working on behalf of AM-Pharma. Data that is pseudonymized (de-identified) means that the Clinical Trial Data given to and used by AM-Pharma is protected by the use of a subject identification number, which is a number specific to you. Only a unique subject identification number for the clinical trial will link the data or samples to you. These data may contain your gender, age, and race, as well as any medical and scientific data required by the clinical trial. Your race and ethnicity will be collected as it is needed to estimate your kidney function, and to assess whether race and ethnicity influence the effects of recAP and how quickly recAP is excreted from the body. The trial doctor maintains a confidential list that links the subject identification number to you. Only the trial doctor will be able to connect the subject identification number to your personal data. He/she will not share this information except as explained in this consent form. The clinical trial site will also establish the necessary technical and organizational measures to prevent your re-identification by AM-Pharma.

<EU ONLY (and only for member states where consent is not considered to be the appropriate legal basis for processing personal data)>: The processing of your personal data is necessary: (i) to comply with AM Pharma's legal and/or regulatory obligations; (ii) for AM Pharma's legitimate interests in conducting research in connection with the clinical trial; (iii) for the performance of a task in the public interest; (iv) when special categories of personal data are processed, for reasons of public interest in the area of public health; and (v) for scientific research.

Data Sharing

<EU ONLY: The trial doctor will transfer information concerning your age, gender, race, and clinical trial site location to a sponsor's service provider established in the United States, for the purpose of assigning your subject identification number. The protection of personal data in the

United States is not as strong as that provided in the European Union; however, appropriate measures will be taken to protect the data >

AM-Pharma, AM-Pharma's representatives, regulatory authorities, or other supervisory bodies may review any pseudonymized Trial Data held by the trial doctor and the clinical trial site. The reason these people may look at your pseudonymized Trial Data is to make sure the clinical trial has been done the right way and that the Trial Data are accurate and adequate for regulatory purposes. A data safety monitoring board has been established to oversee the trial and can access all pseudonymized Trial Data including information about the treatment you received.

The only circumstances in which AM-Pharma, AM-Pharma's representatives, regulatory authorities, data safety monitoring board, or other supervisory bodies may review Trial Data that is not pseudonymized would be where this is necessary to comply with the national law of your country, is necessary for the performance of a task carried out in the public interest, for verification of clinical trial procedures and/or data, and as part of an investigation of an adverse event that occurred during the clinical trial, without violating your confidentiality. All people and organizations, involved in personal data processing who could either directly or indirectly identify your identity, are all obligated to maintain confidentiality by the nature of their work, or are bound by confidentiality agreements.

If you are transferred or readmitted to a hospital other than the trial hospital, your trial doctor or a member of the clinical trial team will continue collecting your personal data as if you stayed at the trial hospital, for the purpose of the clinical trial. The trial hospital will ensure that any collection and transfer of your personal data is done in accordance with applicable laws.

AM-Pharma may share the pseudonymized Trial Data with its representatives, including authorized clinical trial monitors, with other companies within its group, with its service providers, its contractors and business collaborators, and with research institutions and research-based commercial organizations who will use the pseudonymized Trial Data for the purposes described above.

<***Note: this language to be added and adapted as needed to comply with country/site requirements>:*** In certain circumstances (e.g. in the event of medical emergency), AM-Pharma or AM-Pharma's representatives may be performing trial data verification through remote access of medical records. This means someone from the trial staff will provide Sponsor's representatives access to your medical records for only those data points that need verification.

<*Countries outside the EU:* AM-Pharma and those who work for or with AM-Pharma, the IRB/IEC and national and international Regulatory Authorities will be able to see your personal medical files at the clinical trial site, which contain your full name. All people involved in the clinical trial have the duty of confidentiality.>

<*EU ONLY:* Where the national law of <insert Country> so requires, pseudonymized Trial Data may be shared with the competent Ethics Committees overseeing this clinical trial, with [insert details of the competent regulatory authority of the concerned EU Member State], with other

competent regulatory authorities in other EU Member States, with the European Medicines Agency, as well as with the regulatory authorities of non-EU countries, including the U.S. Food and Drug Administration (FDA), the US Department of Health and Human Services, the US National Institutes of Health, and the US Office for Human Research Protections.>

<EU ONLY: You should be aware that non-EEA countries to which your pseudonymized Trial Data will be transferred, e.g., the United States, may not offer the same level of privacy protection as you are used to in the country where you live or where this clinical trial is conducted. However, AM-Pharma will keep any information it receives as confidential as required by applicable local law. AM-Pharma will take reasonable steps necessary to ensure that any Trial Data transferred are treated securely and in accordance with this form to the extent permitted by law. AM-Pharma has also entered into agreements and has, where required by applicable laws, entered into the European Commission approved Standard Contractual Clauses with third parties working with AM-Pharma to ensure the confidentiality of your data and samples. >

<EU ONLY: AM-Pharma B.V. Stadsplateau 6 3521 AZ Utrecht The Netherlands ("AM-Pharma") is responsible for processing your Trial Data. AM-Pharma will act as a controller within the meaning of the General Data Protection Regulation 2016/679 and is required by law to protect your personal data. This section explains how AM-Pharma processes your personal data. <*if required by country:* AM-Pharma's representative for the processing of your personal data in your country is <name and address of the data processing representative from the institution>.

Publication

On completion of the clinical trial, results and data from the trial that will not include any personal identifiers may be published in accordance with regulatory requirements.

Although information about this clinical trial, including the results, may be published for scientific purposes, presented or posted electronically (for example, in a clinical studies registry database) or presented to scientific groups, your name and personal information will not be used and your identity will not be revealed.

<FOR ALL EU SITES AND SITES OUTSIDE THE EU WHERE THIS REQUIREMENT APPLIES should include the specific statement verbatim:> A description of this clinical trial will be available on https://www.clinicaltrialsregister.eu/ as required by EU law. This Web site will not include information that can identify you. At most, the Web site will include a summary of the results. You can search this Web site at any time.

<FOR ALL US SITES AND SITES OUTSIDE THE US WHERE THIS REQUIREMENT APPLIES should include the specific statement verbatim:> A description of this clinical trial will be available on http://www.ClinicalTrials.gov as required by U.S. Law. This Web site will not include information that can identify you. At most, the Web site will include a summary of the results. You can search this Web site at any time.

<FOR ALL US SITES *include HIPAA language or reference to separate HIPAA>*

Storage of Personal Data and Biological Samples

Your personal data and biological samples will be stored for up to five years <*or include requirement for country if applicable* up to X years> after the clinical trial is published. Your samples may be stored for longer than these specified periods if this is required by a regulatory or government agency.

Rights Concerning the Processing of Your Personal Data

You have a right of access to, and, if needed, you have the right to correct your data. You may also have the right to request from sponsor erasure of your personal data, to obtain from AM-Pharma restriction of processing, to object to processing, and to receive personal data in a structured, commonly used and machine-readable format and have it transmitted to another third party provided to sponsor for transfer to a third party (i.e., right to data portability). However, certain personal data collected before you make such a request may need to be processed by AM-Pharma in order to comply with regulations governing clinical research in your Country and cannot, therefore, be erased. You also have the right to withdraw your consent to the processing of your personal data at any time. However, if you withdraw your consent to the processing of your personal data after you have started your participation in the clinical trial this will result in your withdrawal from the clinical trial.

If you have any questions about the collection and use of information about you, or would like to exercise rights that you may have regarding this information, you should ask your trial doctor at [Phone Number]. <***EU Only:*** You also have the right to submit a complaint with <*insert competent data protection authority in the EU Member State Concerned*>.

10. New Information

During the clinical trial, new information about the risks and benefits of the project may become known. Your trial doctor will talk with you about any important new information that is learned during the course of the clinical trial that may affect your willingness to continue to take part in the clinical trial. This new information may also mean that you can no longer take part in this clinical trial. In all cases, you will be offered all available care to suit your needs and/or medical condition.

11. Voluntary Participation/Withdrawal

Taking part in this clinical trial is entirely voluntary. You do not have to take part in this clinical trial and you are free to withdraw at any time. You may withdraw from the clinical trial entirely or you may withdraw only from receiving trial drug and continue trial procedures and/or data collection. If you withdraw entirely, you will be asked to complete an end of trial visit. Unless you withdraw from all future contact, you will be contacted by telephone on Days 28, 90 and 180 to see how you are doing.

If you choose to take part and you change your mind later, you are free to take back your consent and to stop being in the clinical trial (fully or partly) at any time without giving a reason. In that

case, we ask you to tell your trial doctor or a member of the clinical trial team. You may be asked to take part in a final visit or follow-up. If you do take away your consent, no new information or biological samples will be taken and you may also request that no new analysis on your samples will be done.

If you change your mind about allowing your coded biological samples to be used for this clinical trial, contact the trial doctor or a member of the clinical trial team and let them know. You can request destruction of collected samples that would otherwise remain in storage. If you do choose not to have your samples that are required for the clinical trial used, you will no longer be able to take part in this clinical trial.

Your choice to take part or to stop taking part in this clinical trial, will not affect your routine/regular treatment, your relationship with those treating you or your relationship with the place where you are getting treatment. You will still receive care for your condition and will not lose any benefits to which you are otherwise entitled.

12. Premature End of the Clinical Trial or Clinical Trial Treatment

This clinical trial or the trial drug may be stopped without your consent.

Reasons why AM-Pharma can stop the clinical trial or put the clinical trial on hold include:

- recAP has been shown not to work.
- recAP has been shown to work and there is no need for the clinical trial to continue.
- recAP has been shown to cause serious side effects.
- Decisions made in the business or commercial interests of AM-Pharma.
- Decisions made by the Regulatory Authorities or Ethics Committees.

The trial doctor may also stop your treatment with trial drug if it is not in your best interest to continue.

13. Who to contact for more information?

Contacts in case of emergency and for questions about the clinical trial

Please contact a member of the clinical trial team if you have any questions about this clinical trial, its procedures, risks and benefits, or alternative courses of treatment or in case of emergency.

The names and telephone numbers of the clinical trial team to contact are listed in the table below.

Main trial doctor	<NAME>	<CONTACT NUMBER>
Other trial doctor	<NAME>	<CONTACT NUMBER >
Trial nurse	<NAME>	<CONTACT NUMBER >

Contact for questions about your rights

If you have any complaints about any part of the clinical trial, the way it is being done or any questions about your rights as a clinical trial participant, you may contact:
Name of Contact Person: <NAME>
Telephone Number: <NUMBER>
Address: <ADDRESS>

You will receive a card indicating that you are participating in this trial. The card will include the name and phone number of the trial doctor. Please have this card with you at all times, as long as you remain in the trial.

IRB/IEC REVIEW

Any new research studies beyond the current clinical trial using your coded biological samples will be reviewed by the trial doctor's Institutional Review Board (IRB)/Independent Ethics Committee (IEC), a special committee that oversees medical research studies to protect the rights and wellbeing of the patients / healthy volunteers.

***II*. INFORMED CONSENT FORM**

Sign this form ONLY if all of the following statements are true:

- I have read (or someone has read to me) the information in this document in a language that I understand well.
- The content and meaning of this information have been explained to me.
- I have been given an opportunity to ask my questions in private as well as to meet with a member of the clinical trial team to discuss this clinical trial. I have had a chance to consider the information, including the risks and benefits of taking part in this clinical trial, to ask questions, and to discuss the clinical trial. My questions have been answered to my satisfaction.
- I have asked a member of the clinical trial team any questions I may have and have had enough time to decide if I want to take part in this clinical trial.
- I agree that biological samples may be collected from me as explicitly stated in this consent form.
- **I have decided to take part in this clinical trial. I understand I will get a signed and dated copy of this document.**

- *<If applicable:>* I agree that my trial doctor/staff can access my medical records or publicly available records, to determine my health/survival status if contact with me is lost or I withdrew from the trial (initial on the appropriate line).
  ___ Yes ___ No

- *<EU Only (and to only be included where consent is an appropriate legal basis in the Member State):* I agree to the processing and use of my personal data for the purposes of my participation in the clinical trial as described in this information sheet and consent form. I acknowledge that without my consent, my personal data and samples cannot be used and that I will not be able to take part in the clinical trial (initial on the appropriate line)>
  ___ Yes ___ No

- *<EU Only (and to only be included where consent is an appropriate legal basis in the relevant Member State):* I agree to the use of my coded medical information for future medical or pharmaceutical research (initial on the appropriate line)>
  ___ Yes ___ No

- I agree that my primary doctor can be told that I am taking part in this clinical trial (initial on the appropriate line).
  ___ Yes ___ No __ Not applicable, I do NOT have a primary doctor

- *<If applicable:>* I agree to have some trial visits at home to be performed by trial site personnel:
  ___ Yes ___ No __

- *<If applicable:>* I agree to have some trial visits at home to be performed by third party vendor Illingworth Research Group:
  ___ Yes ___ No __

I am free to stop taking part in this clinical trial at any time for any reason and my choice to stop taking part will not affect my future medical care. I agree to follow the clinical trial doctor's instructions and will tell the doctor at once if I have any changes in my health. By signing this document, I am not giving up any of my legal rights.

Printed Name of Patient	
Signature of Patient	*Date and time of Signature*
Signature of Legal Authorized Representative (if applicable)	*Date and time of Signature*
Signature of impartial witness (if applicable)	*Date and time of Signature*

I, the undersigned, trial doctor/investigator/ clinical trial personnel, confirm that I have verbally given the necessary information about the clinical trial, that I answered any additional questions, and that I did not exert any pressure on the patient to participate in the clinical trial.

I declare that I acted in full accordance with the ethical principles described in GCP Guidelines and other national and international legislation in effect.

A copy of this patient information sheet and consent form, signed by both parties, will be provided to the patient.

Printed Name of Person Obtaining Consent	
Signature of Person Obtaining Consent	*Date and time of Signature*

Incapacitated patients enrolled in the clinical trial must sign the most recent ICF as soon as they are capable to do so.