

BMJ Open 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.

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.

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.

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NCT04411472.

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 $\mu\text{g}/\text{kg}/\text{min}$ 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.

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 $\mu\text{g}/\text{kg}/\text{min}$ 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 $\mu\text{mol}/\text{L}$) 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 $\text{mL}/\text{kg}/\text{hour}$ 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 $\text{mL}/\text{min}/1.73$ 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 $\text{mL}/\text{min}/1.73$ m^2 and < 45 $\text{mL}/\text{min}/1.73$ 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

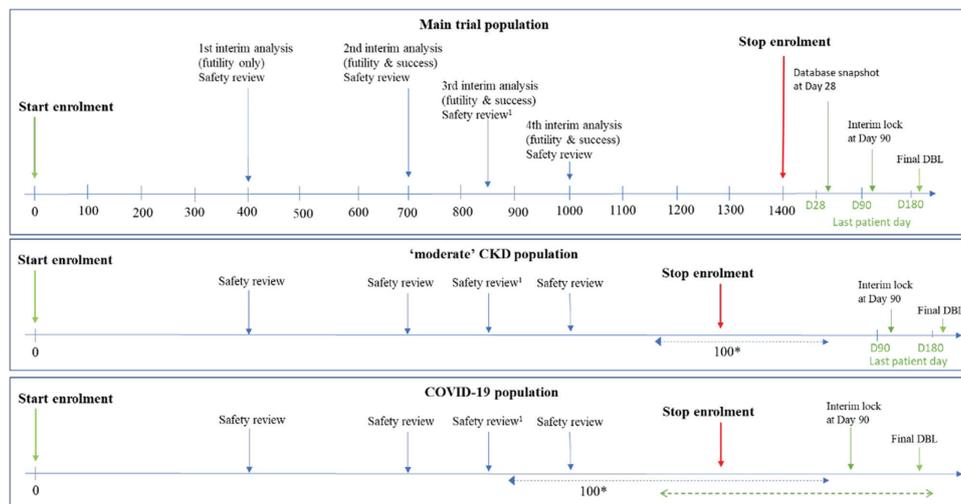


Figure 1 Trial design. Up to 1400 patients in the main trial population, up to approximately 100 patients in the ‘moderate to severe’ CKD population and up to approximately 100 patients in the COVID-19 population will be enrolled. There will be a maximum of four interim analyses, after approximately 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 concerns¹. 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. ¹A 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/exploratory 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

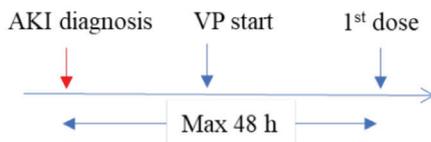
acceptance of the manuscript, an amended SAP was written and approved prior to the first interim-analysis. This amendment, which were not subjected to peer-review, is depicted in [online supplemental file 4](#).

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

Timelines for eligibility

1. When AKI is diagnosed *before* start of vasopressor therapy:



2. When AKI is diagnosed *after* start of vasopressor therapy:

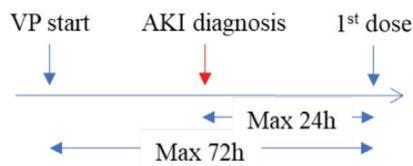


Figure 2 Timelines for eligibility. To be eligible for the trial, patients must have both sepsis requiring vasopressor therapy and AKI. (1) When AKI is diagnosed before the start of vasopressor therapy, infusion of first dose of trial drug must start within 48 hours of AKI diagnosis. (2) When AKI is 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 $\mu\text{g}/\text{kg}/\text{min}$ 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-AKI²⁹ 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 alpha 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 $\geq 25\%$ 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

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 $\geq 25\%$ 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 $\geq 25\%$ 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-Dimension-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/exploratory 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

(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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Contributors PP and ALK co-drafted the manuscript, participated in the trial protocol design and are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. DCA, JA, RB, EvdB, JB, MB, KB, JC, CJD, RF, MJ, BF, KD, JAK, P-FL, KL, RLM, PTM, MO, VP, SR, PY and AZ all participated in the trial protocol design (as members of the trial steering committee (TSC), reviewed and edited the manuscript. All authors read and approved the final version of the manuscript prior to submission.

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