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Fortsættes ...

DESCRIPTION

Description

BACKGROUND

[0001] The kidneys perform several functions in an animal body, such as excretion of waste, acid-base homeostasis, osmolality regulation, blood pressure regulation and hormone secretion. To enable the kidneys to perform these tasks, the kidneys receive, despite their relatively small size, approximately 20% of the cardiac output. Consequently a disruption of blood flow to the kidneys (renal blood flow, RBF) has a direct impact on many of the functions of the kidney. For instance, reduction of excretion of nitrogenous waste and disturbances of fluid and electrolyte balances could then occur. On the long term, reduced RBF, but also other toxic events, such as ischemia (reperfusion injury), use of contrast media or (other) nephrotoxic drugs, e.g. antibiotics, can be so stressful to the kidneys that it results in acute kidney injury (AKI).

[0002] Acute Kidney Injury (AKI) is observed in up to 60% of patients in intensive care units (ICUs), and its incidence is increasing (Hoste et al. Intensive Care Med 2015;41:1411-23; Hoste et al. Critical Care; 10; Nisula et al. Intensive Care Med 2013;39:420-8; Vincent et al. Critical Care Medicine 2006;34:344-53). Development of AKI in sepsis patients is associated with an increased mortality (Kellum et al. Critical Care Medicine 2016;193:281-7), while survivors are at risk of developing chronic kidney disease (Chawla et al. Kidney Int 2011;79:1361-9; Oppert et al. Nephrol Dial Transplant 2008;23:904-9; Vaara et al. Crit Care 2012;16:R197) resulting in a tremendous burden for both patient and society.

[0003] It is very important to prevent and/or adequately treat AKI, because first, AKI is accompanied with high costs, morbidity and mortality, and second, prolonged AKI may lead to chronic kidney injury (CKI), possibly leading to irreversible loss of renal function. Additionally, an individual that has acquired CKI is more prone to (again) acquire AKI, which is then called "acute on chronic kidney injury". Because the renal function in CKI patients is already reduced, acute on chronic kidney injury may reduce renal function below a critical threshold.

BRIEF SUMMARY

[0004] The present disclosure provides an alkaline phosphatase (AP) for use in a method to treat sepsis-associated acute kidney disease injury (SA-AKI) in a subject in need thereof, comprising administering an effective amount of alkaline phosphatase (AP) to said subject,

wherein (i) the subject has an eGFR (estimated glomerular filtration rate) ≤ 60 ml/min prior to the treatment with AP, (ii) the AP is administered in at least one 500 U/kg to 2,000 U/kg dose, and (iii) the administration of the AP results in an increase in renal function.

[0005] In some aspects, the subject has 15-60 ml/min eGFR prior to treatment with AP. In some aspects, the subject has <15 ml/min eGFR prior to treatment with AP. In some aspects, the AP is a human AP. In some aspects, the AP is a recombinant AP. In some aspects, the recombinant AP is chimeric. In some aspects, the chimeric AP has at least at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to RecAP (SEQ ID NO: 1).

[0006] In some aspects, the increase in renal function comprises an increase in endogenous creatinine clearance (ECC) with respect to ECC in the absence of treatment or an increase in eGFR with respect to eGFR in the absence of treatment. In some aspects, sepsis is detected less than 96 hours prior to AP administration. In some aspects, the sepsis is detected less than 72 hours prior to SA-AKI detection.

[0007] In some aspects, treatment is initiated within 24 hours after sepsis is detected. In some aspects, treatment is initiated within 24 hours after SA-AKI is detected. In some aspects, AP is administered once daily. In some aspects, AP is administered intravenously. In some aspects, AP is administered in three daily doses. In some aspects, the AP dose is 0.8 mg/kg or 1.6 mg/kg of RecAP, e.g., the clinical grade RecAP used in the present disclosure. In some aspects, the AP dose is 500 U/kg or 1000 U/kg of RecAP, e.g., the clinical grade RecAP used in the present disclosure.

[0008] In some aspects, the administration of at least one dose of AP results in a shortening of duration or cessation of renal replacement therapy (RRT) in a subject undergoing RRT. In some aspects, the administration of at least one dose of AP results in the preservation or increase of glomerular filtration rate (GFR) or eGFR in the subject.

BRIEF DESCRIPTION OF THE DRAWINGS/FIGURES

[0009]

FIG. 1 is a table showing proposed RecAP dose groups.

FIG. 2 shows a RecAP amino acid sequence (SEQ ID NO: 1).

FIG. 3 shows a timeline of the STOP-AKI study, including dosage regimen and timeframes to determine AUC_{1-7} , RRT incidence, and mortality and MAKE.

FIG. 4 is a flowchart showing the enrollment, randomization, and follow-up of the patients in the trial. Abbreviations: AKI (acute kidney injury), AUC (time-corrected area under the curve), ECC (endogenous creatinine clearance), ICF (informed consent form), ITT (intention-to-treat),

IV (intravenous), MAKE (major adverse kidney events), NS (not specified), RecAP (human recombinant alkaline phosphatase having the sequence of SEQ ID NO: 1), RRT (renal-replacement therapy), SA-AKI (sepsis-associated acute kidney injury), SCr (serum creatinine).

FIG. 5 is a table presenting the demographic and baseline characteristics of all groups in the trial, showing that there were no significant differences between groups in any baseline characteristics. For variables with missing data, summary data are based on the adjusted number. Body-mass index (BMI) is the weight in kilograms divided by the square of the height in meters. Scores on the Acute Physiology and Chronic Health Evaluation II (APACHE II) range from 0-71, with higher scores indicating greater disease severity. Scores on the Sequential Organ Failure Assessment (SOFA) range from 0-24, with higher scores indicating more severe dysfunction. Scores on the Simplified Acute Physiology Score (SAPS) range from 0-163 with higher scores indicating greater disease severity. Estimated glomerular filtration rate (eGFR) was calculated according to the CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration) equation: $141 \cdot \min(\text{Scr}/\kappa, 1)^\alpha \cdot \max(\text{Scr}/\kappa, 1)^{-1.209} \cdot 0.993^{\text{Age}} \cdot 1.018[\text{if female}] \cdot 1.159[\text{if black}]$. Scr is serum creatinine (mg/dL), κ is 0.7 for females and 0.9 for males, α is -0.329 for females and -0.411 for males, min indicates the minimum of Scr/ κ or 1, and max indicates the maximum of Scr/ κ or 1. (www.kidney.org/content/ckd-epi-creatinine-equation-2009). Acute Kidney Injury (AKI) stage was stratified according to the AKI-Network definition (www.akinet.org/akinstudies.php). Median and interquartile values, or number and percentages are depicted.

FIG. 6 is an AUC vs. dose graph showing a linear increase in RecAP concentrations. Exposure in patients in the STOP-AKI clinical trial is slightly higher compared to healthy subjects. Dose linearity and proportionality were observed over the dose range (0.4 - 1.6 mg/kg).

FIG. 7 is a box whisker plot showing creatinine clearance AUC_{1-7} values corresponding to placebo, and RecAP doses of 0.4 mg/kg, 0.8 mg/kg, and 1.6 mg/kg. The values plotted are shown in the table under the plot. AUC_{1-7} denotes area under the ECC curve from day 1 through day 7, divided by 7 to provide a time-corrected clearance in ml/min.

FIG. 8 is a table showing primary and secondary end points (placebo and RecAP 0.6 mg/kg group). AUC_{1-7} denotes area under the ECC curve from day 1 through day 7, divided by 7 to provide a time-corrected clearance in ml/min. Abbreviations: CI (confidence interval), CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration), ECC (endogenous creatinine clearance), eGFR (estimated glomerular filtration rate), ICU (intensive care unit), IQR (interquartile range), MAKE (major adverse kidney events), RRT (renal-replacement therapy), SOFA (Sequential Organ Failure Assessment).

§ RRT incidence indicates the percentage of patients that needed RRT following randomization.

|| With last observation carried forward.

* MAKE 28: received RRT before or on day 28 or died before or on day 28. Proportion that met at least one of the criteria.

† MAKE 60: eGFR <60 ml/min at day 60 estimated by the CKD-EPI formula based on serum creatinine or required chronic RRT, before or on day 60 or died before or on day 60. Proportion that met at least one of the criteria.

‡ MAKE 90: 60: eGFR <60 ml/min at day 90 estimated by the CKD-EPI formula based on serum creatinine or required chronic RRT, before or on day 90 or hospitalization for new episode of AKI before or on day 90 or died before or on day 90. Proportion that met at least one of the criteria.

§§ Denotes the absolute difference (for continuous variables).

†† Denotes the odds ratio (for categorical variables).

‡‡ Denotes the hazard ratio (for events).

FIG. 9A shows the effect of RecAP on Endogenous Creatinine Clearance (ECC). Treatment effect of RecAP compared with placebo on ECC total curve: $P=0.036$. For missing values last observation carried forward (LOCF) and interpolation was applied. * depicts $P<0.05$., # depicts $P=0.06$.

FIG. 9B shows the effect of RecAP on Blood Urea Nitrogen (BUN) clearance. Treatment effect of RecAP compared with placebo on BUN clearance total curve: $P=0.033$. For missing values last observation carried forward (LOCF) and interpolation was applied. * depicts $P<0.05$., # depicts $P=0.06$.

FIG. 10 shows a Kaplan-Meier probability of survival curve for SA-AKI subjects from randomization to day 90 (placebo group and RecAP 1.6 mg/kg group). Effect of RecAP compared with placebo on day 28 mortality: $P=0.02$. Effect of RecAP compared with placebo on day 90 mortality: $P=0.03$.

FIG. 11 shows probability of survival for SA-AKI subjects after administration of RecAP at 0.4 mg/kg, 0.8 mg/kg, or 1.6 mg/kg doses, or placebo over 90 days.

FIG. 12 shows primary and secondary endpoints for all treatment groups (RecAP at 0.4 mg/kg, 0.8 mg/kg, or 1.6 mg/kg doses, or placebo).

FIG. 13A shows quality of life (QoL) assessments measured by EuroQoL (EQ5D) score at ICU (left drawing) and Day 90 (right drawing).

FIG. 13B shows lung function parameters (P/F ratio, tidal volume and PEEP), depicted as medians, for the 1.6 mg/kg RecAP dose and placebo groups. Numeric values are shown below the graphics.

FIG. 13C shows liver function parameters (alanine aminotransferase, and aspartate aminotransferase activity), depicted as medians, for the 1.6 mg/kg RecAP dose and placebo groups. Numeric values are shown below the graphics.

FIG. 13D shows liver function parameters (gamma-glutamyl transferase activity, and bilirubin

levels), depicted as medians, for the 1.6 mg/kg RecAP dose and placebo groups. Numeric values are shown below the graphics.

FIG. 13E shows LDH activity, depicted as median, for the 1.6 mg/kg RecAP dose and placebo groups. Numeric values are shown below the graphics.

FIG. 14 shows the results of a post hoc multivariate analysis.

FIG. 15A shows C-reactive protein (CRP) concentrations (mg/L), depicted as medians, for the 1.6 mg/kg RecAP dose and placebo groups. Numeric values are shown to the right of the graphic.

FIG. 15B shows interleukin 6 (IL-6) concentrations (pg/ml), depicted as medians, for the 1.6 mg/kg RecAP dose and placebo groups. Numeric values are shown to the right of the graphic.

FIG. 15C shows interleukin 18 (IL-18) concentrations (pg/ml), depicted as medians, for the 1.6 mg/kg RecAP dose and placebo groups. Numeric values are shown to the right of the graphic.

FIG. 15D shows lipopolysaccharide binding protein (LBP) concentrations (µg/ml), depicted as medians, for the 1.6 mg/kg RecAP dose and placebo groups. Numeric values are shown to the right of the graphic.

FIG. 15E shows Kidney Injury Molecule 1 (KIM-1) concentrations (pg/ml), depicted as medians, for the 1.6 mg/kg RecAP dose and placebo groups. Numeric values are shown to the right of the graphic.

FIG. 15F shows the influence of the duration between first diagnosis of AKI and start of study drug infusion (hours) on primary endpoint ECC.

FIG. 15G shows the influence of baseline disease severity by APACHE II score on primary endpoint ECC.

FIG. 15H shows the influence of inflammation as measured by baseline C-reactive protein (CRP) concentrations (mg/L) on primary endpoints ECC.

FIG. 15I shows the influence of inflammation as measured by baseline interleukin 6 (IL-6) concentrations (pg/ml) on primary endpoint ECC.

FIG. 15J shows the influence of the fractional excretion of sodium (% of FeNa) at baseline on primary endpoint ECC.

FIG. 15K shows the influence of baseline Kidney Injury Molecule 1 (KIM-1) concentrations (pg/ml) on primary endpoint ECC.

FIG. 15L shows the difference in baseline kidney function on primary endpoint ECC, depicted as medians.

FIG. 16 shows treatment-emergent adverse events observed during the STOP-AKI study.

FIG. 17 shows a subgroup analysis of subjects in the STOP-AKI study stratified according to severity in kidney injury. Thresholds or cut-offs are based on ECC.

FIG. 18 shows coefficients and model fit statistics of two different Cox parallel-hazard models (CoxPH). (Model 1) CoxPH model that predicts overall survival (OS) in the STOP-AKI study as a continuous function of the baseline ECC, where the measurements at baseline and day 1 were averaged (MeanBaseECC; missing values were ignored in this calculation). (Model 2) CoxPH model predicting OS in 3 different groups on the basis of MeanBaseECC as follows; <15 as the reference group, >=15 but <60 as the Severe-Mild group and >= 60 as the Normal group.

FIG. 19 shows hazard ratios (HR) for overall survival according to the continuous CoxPH model (see Model 1 in **FIG. 18**) as a function of average baseline ECC, where measurements at baseline and day 1 were averaged. The HR relative to the mean ECC in the population, 46 ml/min, was calculated according to the model at different baseline levels (straight line) together with the standard errors (dashed line) and compared to the reference level, an HR of 1 (dotted horizontal reference line).

FIG. 20 shows hazard ratios (HR) for overall survival according to the continuous CoxPH model (see Model 1 in **FIG. 18**) at different values of average baseline ECC in ml/min, where measurements at baseline and day 1 were averaged. For each group according to CKD criteria, the median of average baseline ECC is tabulated, together with the number of patients in each group, and the HR for OS relative to the mean average baseline ECC, 46 ml/min, according to the CoxPH model and its standard error (seHR). Additionally, the CKD criteria boundaries, i.e., 15 ml/min between "Kidney failure" (Severe Kidney Injury) and "Severe-mild" (Moderate to Severe Kidney Injury), and subsequently 60 ml/min as the boundary to "Normal" (Mild Injury or Normal) are tabulated with corresponding model HR and standard error.

FIG 21 shows a Kaplan Meier Time to Death curve (per baseline eGFR subgroups). Baseline eGFR was measured with CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration) equation.

FIG 22 shows a Kaplan Meier Time to Death curve (recAP versus placebo in subgroups with baseline eGFR above (right panel) and below (left panel) 60 mL/min). Baseline eGFR was measured with CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration) equation.

DETAILED DESCRIPTION

[0010] Sepsis-associated AKI (SA-AKI) is a multifactorial syndrome with inflammatory, nephrotoxic, and ischemic insults occurring simultaneously with other pathophysiological responses rapidly leading to renal impairment (Bonventre et al. J Clin Invest 2011;121:4210-21; Gomez et al. Shock 2014;41:3-11). Currently, no approved pharmacologic interventions are available to prevent or treat AKI (Joannidis et al. Intensive Care Medicine 2017;43:730-49).

Alkaline phosphatase (AP) was originally considered as a novel treatment for sepsis in general (Poelstra et al. American Journal of Pathology 1997;151:1163-69.; Verweij et al. Shock 2004;22:174-9). In two small clinical trials bovine AP administration improved renal function in patients with sepsis (Heemskerk et al. Crit Care Med 2009;37:417-23, e1; Pickkers et al. Crit Care 2012;16:R14).

[0011] The STOP-AKI clinical trial was an international randomized, double-blind, placebo-controlled, four arm, parallel-group, dose-finding adaptive phase IIa/IIb multicenter study conducted in critically ill adults with sepsis-associated acute kidney injury. In this study a total of 301 patients were enrolled across 53 sites in 11 countries in the European Union and North America. The four arms of the study consisted of patients receiving RecAP 0.4 mg/kg, 0.8 mg/kg, 1.6 mg/kg or placebo. Following the interim-analysis, the study continued with RecAP 1.6 mg/kg as treatment.

[0012] RecAP is a chimeric AP that combines the properties of two human isoenzymes, intestinal and placental AP (Kiffer-Moreira et al. PLoS One 2014;9:e89374). Replacing the crown domain of intestinal AP (the most biologically active isoenzyme) with the crown domain of placental AP (which has the longest half-life) creates a highly stable, biologically active enzyme (Kiffer-Moreira et al. PLoS One 2014;9:e89374). See, e.g., U.S. Patent Nos. US8586032 and US8557545, and U.S. Patent Appl. Publ. Nos. US20170009216 and US20160250299.

[0013] Compared with placebo, RecAP 1.6 mg/kg did not affect creatinine clearance in the first week or renal replacement therapy rates. However, improvement of endogenous creatinine clearance on days 21 ($P=0.02$) and 28 ($P=0.01$) was more pronounced and the incidence of major adverse kidney events at days 60 ($P=0.045$) and 90 ($P=0.03$) was lower in patients receiving RecAP. All-cause 28-day mortality was 14.4% in RecAP-treated patients versus 26.7% in placebo-treated patients ($P=0.02$). No significant differences in (serious) adverse events were observed between groups. RecAP appeared safe and well tolerated.

[0014] A key observation of the study was that although short-term renal function was not affected, unexpected long-term beneficial effects derived from RecAP treatment were observed. These unexpected observation showed that treatment with RecAP resulted in enhanced and sustained recovery of renal function and better survival compared to placebo.

[0015] The long-term effects varied depending on the severity of the impairment in kidney function, i.e., some specific subgroups of subject responded particularly well to AP therapy. Thus, stratifying patients according to, e.g., particular markers of kidney function and/or biomarkers (e.g., ECC or eGFR) allows to identify particular effects of AP administration on specific subgroups. The parameter or parameters defining each of these subgroups (e.g., a series of thresholds, such as ECC or eGFR thresholds) can be used, for example, to evaluate the likelihood of a positive outcome, to selected patients for treatment, or to make decisions related to the AP treatment (e.g., modify dosage). For example, if the subject had moderate to severe kidney injury (corresponding, e.g., to a 15 mg/ml to 60 ml/min ECC), there was a

statistically significant effect of RecAP which resulted in improved kidney function (as evidenced by improved creatinine clearance with respect to placebo). If the subject had severe kidney injury (corresponding, e.g., to <15 ml/min baseline ECC), there was a statistically significant long term effect resulting in improved survival. If the subject had ≤ 60 eGFR, there was also a statistically significant long term effect resulting in improved survival.

[0016] A goal of the present disclosure is to provide alkaline phosphatase (AP) for use in methods for preserving or improving renal function in a subject who suffers from sepsis-associated acute kidney disease injury (SA-AKI). Another goal of the present disclosure is to prevent or shorten the duration of renal-replacement therapy (RRT), to increase the kidney creatinine clearance, and/or to increase survival.

[0017] Accordingly, the present disclosure relates to an alkaline phosphatase (AP), e.g., RecAP, for use in methods to preserve or improve kidney function in a subject with sepsis-associated AKI (SA-AKI). The disclosure provides, for example, alkaline phosphatase (AP) for use in methods for treating a subject with SA-AKI, comprising administering an AP, e.g., RecAP, to the subject. In some aspects, each dose of AP administered to the subject comprises at least 500 U/kg (0.8 mg/kg in the case of the clinical grade RecAP used). In some particular aspects, each AP dose is at least 1,000 U/g (1.6 mg/kg in the case of the clinical grade RecAP used).

[0018] In some aspects, the AP, e.g., RecAP, is administered to the patient if SA-AKI, is present and the kidney injury has been determined to be moderate to severe, or severe. Thus, in some aspects of the present disclosure, the AP for use in methods disclosed herein comprise measuring severity of kidney injury, e.g., by determining glomerular filtration rate or a surrogate measure such as eGFR, prior to treatment with AP to determine whether the subject suffers moderate to severe kidney injury, or severe kidney injury.

[0019] Diagnostic measurements can be used to determine the severity of the kidney injury, e.g. eGFR. In some aspects of the present disclosure, AP treatment is not administered to the subject if kidney injury is determined to be mild injury (e.g., if >60 ml/min baseline eGFR. As can be appreciated by the person skilled in the art, ECC and eGFR are correlated and a person having an ECC of <15 ml/min, for instance, is likely to (also) have an eGFR of <15 ml/min as well and vice versa. The same holds true for an ECC of > 60 ml/min in respect of eGFR of >60 ml/min and an ECC of between 15 - 60 ml/min in respect of eGFR of between 15 - 60 ml/min. Nevertheless, there may be some differences between ECC and eGFR values and eGFR is used for determining whether a patient may or may not benefit from administration of AP.

[0020] The AP for use in methods disclosed herein comprise administering at least one dose of AP, e.g., RecAP, to the subject wherein each dose is least 500 U/kg (0.8 mg/kg for the clinical grade RecAP used) or 1000 U/kg (1.6 mg/kg for the clinical grade RecAP used). In some specific aspects, the dosage regimen comprises administering 0.5 mg/kg to 2 mg/kg of an AP, e.g., RecAP, by intravenous infusion, in daily doses, for at least three days. In some

specific aspects, the dosage regimen comprises administering 0.5 mg/kg to 2 mg/kg of an AP, e.g., RecAP, by intravenous infusion, in daily doses, for at least three days.

[0021] In order that the present disclosure can be more readily understood, certain terms are first defined. Additional definitions are set forth throughout the detailed description.

I. Definitions

[0022] In this specification and the appended claims, the singular forms "a", "an" and "the" include plural referents unless the context clearly dictates otherwise. The terms "a" (or "an"), as well as the terms "one or more," and "at least one" can be used interchangeably herein.

[0023] Furthermore, "and/or" where used herein is to be taken as specific disclosure of each of the two specified features or components with or without the other. Thus, the term "and/or" as used in a phrase such as "A and/or B" herein is intended to include "A and B," "A or B," "A" (alone), and "B" (alone). Likewise, the term "and/or" as used in a phrase such as "A, B, and/or C" is intended to encompass each of the following aspects: A, B, and C; A, B, or C; A or C; A or B; B or C; A and C; A and B; B and C; A (alone); B (alone); and C (alone).

[0024] Wherever aspects are described herein with the language "comprising," otherwise analogous aspects described in terms of "consisting of" and/or "consisting essentially of" are also provided.

[0025] The term "about" as used in connection with a numerical value throughout the specification and the claims denotes an interval of accuracy, familiar and acceptable to a person skilled in the art. In general, such interval of accuracy is $\pm 15\%$.

[0026] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure is related.

[0027] Units, prefixes, and symbols are denoted in their Système International de Unites (SI) accepted form.

[0028] Numeric ranges are inclusive of the numbers defining the range. Where a range of values is recited, it is to be understood that each intervening integer value, and each fraction thereof, between the recited upper and lower limits of that range is also specifically disclosed, along with each subrange between such values. The upper and lower limits of any range can independently be included in or excluded from the range, and each range where either, neither or both limits are included is also encompassed within the invention. Where a value is explicitly recited, it is to be understood that values which are about the same quantity or amount as the recited value are also within the scope of the invention. Where a combination is disclosed, each subcombination of the elements of that combination is also specifically disclosed and is

within the scope of the invention. Conversely, where different elements or groups of elements are individually disclosed, combinations thereof are also disclosed. Where any element of an invention is disclosed as having a plurality of alternatives, examples of that invention in which each alternative is excluded singly or in any combination with the other alternatives are also hereby disclosed; more than one element of an invention can have such exclusions, and all combinations of elements having such exclusions are hereby disclosed.

[0029] As used herein the terms "treat," "treatment," or "treatment of" refers to (i) reducing the potential or risk for a disease or disorder, e.g., AKI (e.g., SA-AKI), (ii) reducing the occurrence of a disease or disorder, e.g., AKI (e.g., SA-AKI), (iii) reducing the severity (e.g., ameliorating the symptoms) of a disease or disorder, e.g., AKI (e.g., SA-AKI), or (iv) a combination thereof.

[0030] For example, treating can refer to the ability of a therapy when administered to a subject, to prevent or reduce the risk of kidney injury, and/or to cure or to alleviate symptoms, signs, or causes of kidney injury. Treating also refers to mitigating or decreasing at least one clinical symptom and/or inhibition or delay in the progression of the condition and/or prevention or delay of the onset of a disease or illness when compared with a non-treated (or placebo treated) group. Thus, the terms "treat," "treating" or "treatment of" (or grammatically equivalent terms) refer to both prophylactic and therapeutic treatment regimes. In case of renal disease, it is preferred that subjects, after being treated with an AP, improve in renal function or less decline in renal function, relative to subjects that have not been treated with said AP.

[0031] As used herein, the term "preserving" includes preventing a reduction, slowing down a reduction, stopping a reduction and/or at least partly reversing a reduction of a renal function. The term "increasing" is not necessarily limited to increasing said renal function to a value equal to or higher than that before said treatment occurred. It includes partly restoring renal function.

[0032] The term "treatment with a risk of decreasing renal function" is typically used to refer to a treatment which bears the risk that renal function is reduced by said treatment when comparing the value of at least one renal related parameter to a recognized or average (laboratory) value of said parameter, or by comparing said parameter to the value before said treatment is performed. If, for example, the amount of protein in the urine of a subject, preferably a human being is significantly above a recognized or average (laboratory) value, said renal function is said to be "decreased." The corresponding analysis can be performed in a laboratory but also in a home setting. For example, since September 2006 the Dutch "Nierstichting" has introduced a simple test (named Kidney check ("Niercheck")) which can be performed at home to test whether the kidneys function properly. This test is for instance directed to the amount of protein in the urine.

[0033] The terms "subject" or "patient" as used herein refer to any subject, particularly a mammalian subject, for whom therapy or prognosis of SA-AKI is desired. As used herein, the terms "subject" or "patient" include any human or nonhuman animal. As used herein, phrases such as "a patient having SA-AKI" includes subjects, such as mammalian subjects, that would

benefit from the administration of a therapy with AP, as disclosed herein.

[0034] In some aspects of the present disclosure, a subject is a naive subject. A naive subject is a subject that has not been administered a therapy, for example a therapeutic agent. In some aspects, a naïve subject has not been treated with a therapeutic agent prior to being diagnosed with SA-AKI.

[0035] In another aspect, a subject has received therapy and/or one or more doses of a therapeutic agent prior to being diagnosed as having SA-AKI.

[0036] In some aspects, a subject can be administered at least one therapeutically effective dose of an AP, e.g., RecAP, if the subject's baseline eGFR is below a predetermined threshold level, or if the baseline eGFR is above a predetermined eGFR threshold level, or if the baseline eGFR is within a predetermined range.

[0037] The terms "therapeutic agent" and "drug" as used herein also refer to any therapeutically active substance that is administered to a subject having a disease or disorder, e.g., kidney injury, e.g., SA-AKI, or a disease or condition (e.g., sepsis) that can lead to kidney injury to produce a desired, usually beneficial, effect. A therapeutic agent can also be a prodrug, which metabolizes into the desired therapeutically active substance when administered to a subject. In some aspects, the therapeutic agent is a prophylactic agent. In addition, a therapeutic agent can be pharmaceutically formulated. A therapeutic agent can also be or comprise a radioactive isotope or agent activated by some other form of energy such as light or ultrasonic energy, or by other circulating molecules that can be systemically administered.

[0038] In some aspects of the present disclosure, a therapeutic agent for the treatment, prevention, or amelioration of the symptom of SA-AKI comprises an AP, e.g., RecAP; alone or in combination with one or more standard therapeutic agents generally used for the treatment of kidney injury, e.g., SA-AKI, or a disease or condition (e.g., sepsis) that can lead to kidney injury.

[0039] A "therapeutically effective" amount as used herein is an amount of therapeutic agent that provides some improvement or benefit to a subject having SA-AKI. Thus, a "therapeutically effective" amount is an amount that provides some alleviation, mitigation, and/or decrease in at least one clinical symptom of SA-AKI,.

[0040] Clinical symptoms associated with SA-AKI are well known to those skilled in the art. Further, those skilled in the art will appreciate that the therapeutic effects need not be complete or curative, as long as some benefit is provided to the subject. In some aspects, the term "therapeutically effective" refers to an amount of a therapeutic agent therapeutic agent that is capable of altering biomarker levels, e.g., ECC or eGFR in a SA-AKI patient in need thereof.

[0041] As used herein, a "sufficient amount" or "an amount sufficient to" achieve a particular

result in a patient having SA-AKI refers to an amount of a therapeutic agent (e.g., an AP such as RecAP) that is effective to produce a desired effect, which is optionally a therapeutic effect (i.e., by administration of a therapeutically effective amount). In some aspects, such particular result is an improvement in kidney function or an increase in survival.

[0042] As used herein, the term "healthcare provider" refers to individuals or institutions that directly interact and administer to living subjects, e.g., human patients. Non-limiting examples of healthcare providers include doctors, nurses, technicians, therapist, pharmacists, counselors, alternative medicine practitioners, medical facilities, doctor's offices, hospitals, emergency rooms, clinics, urgent care centers, alternative medicine clinics/facilities, and any other entity providing general and/or specialized treatment, assessment, maintenance, therapy, medication, and/or advice relating to all, or any portion of, a patient's state of health, including but not limited to general medical, specialized medical, surgical, and/or any other type of treatment, assessment, maintenance, therapy, medication and/or advice.

[0043] As used herein, the term "clinical laboratory" refers to a facility for the examination or processing of materials derived from a living subject, e.g., a human being. Non-limiting examples of processing include biological, biochemical, serological, chemical, immunohematological, hematological, biophysical, cytological, pathological, genetic, or other examination of materials derived from the human body for the purpose of providing information, e.g., for the diagnosis, prevention, or treatment of any disease or impairment of, or the assessment of the health of living subjects, e.g., human beings. These examinations can also include procedures to collect or otherwise obtain a sample, prepare, determine, measure, or otherwise describe the presence or absence of various substances in the body of a living subject, e.g., a human being, or a sample obtained from the body of a living subject, e.g., a human being.

[0044] As used herein, the term "healthcare benefits provider" encompasses individual parties, organizations, or groups providing, presenting, offering, paying for in whole or in part, or being otherwise associated with giving a patient access to one or more healthcare benefits, benefit plans, health insurance, and/or healthcare expense account programs.

[0045] In some aspects, a healthcare provider can administer or instruct another healthcare provider to administer a therapy to prevent, treat, or ameliorate the symptoms of SA-AKI. A healthcare provider can implement or instruct another healthcare provider or patient to perform the following actions: obtain a sample, process a sample, submit a sample, receive a sample, transfer a sample, analyze or measure a sample, quantify a sample, provide the results obtained after analyzing/measuring/quantifying a sample, receive the results obtained after analyzing/measuring/quantifying a sample, compare/score the results obtained after analyzing/measuring/quantifying one or more samples, provide the comparison/score from one or more samples, obtain the comparison/score from one or more samples, administer a therapy (e.g., an AP such as RecAP), commence the administration of a therapy, cease the administration of a therapy, continue the administration of a therapy, temporarily interrupt the administration of a therapy, increase the amount of an administered therapeutic agent,

decrease the amount of an administered therapeutic agent, continue the administration of an amount of a therapeutic agent, increase the frequency of administration of a therapeutic agent, decrease the frequency of administration of a therapeutic agent, maintain the same dosing frequency on a therapeutic agent, replace a therapy or therapeutic agent by at least another therapy or therapeutic agent, combine a therapy or therapeutic agent with at least another therapy or additional therapeutic agent.

[0046] In some aspects, a healthcare benefits provider can authorize or deny, for example, collection of a sample, processing of a sample, submission of a sample, receipt of a sample, transfer of a sample, analysis or measurement a sample, quantification a sample, provision of results obtained after analyzing/measuring/quantifying a sample, transfer of results obtained after analyzing/measuring/quantifying a sample, comparison/scoring of results obtained after analyzing/measuring/quantifying one or more samples, transfer of the comparison/score from one or more samples, administration of a therapy or therapeutic agent, commencement of the administration of a therapy or therapeutic agent, cessation of the administration of a therapy or therapeutic agent, continuation of the administration of a therapy or therapeutic agent, temporary interruption of the administration of a therapy or therapeutic agent, increase of the amount of administered therapeutic agent, decrease of the amount of administered therapeutic agent, continuation of the administration of an amount of a therapeutic agent, increase in the frequency of administration of a therapeutic agent, decrease in the frequency of administration of a therapeutic agent, maintain the same dosing frequency on a therapeutic agent, replace a therapy or therapeutic agent by at least another therapy or therapeutic agent, or combine a therapy or therapeutic agent with at least another therapy or additional therapeutic agent.

[0047] In addition a healthcare benefits provides can, e.g., authorize or deny the prescription of a therapy, authorize or deny coverage for therapy, authorize or deny reimbursement for the cost of therapy, determine or deny eligibility for therapy, etc.

[0048] In some aspects, a clinical laboratory can, for example, collect or obtain a sample, process a sample, submit a sample, receive a sample, transfer a sample, analyze or measure a sample, quantify a sample, provide the results obtained after analyzing/measuring/quantifying a sample, receive the results obtained after analyzing/measuring/quantifying a sample, compare/score the results obtained after analyzing/measuring/quantifying one or more samples, provide the comparison/score from one or more samples, obtain the comparison/score from one or more samples, or other related activities.

II. Treatment of Acute Kidney injury with AP

[0049] In certain aspects, the present disclosure is related to alkaline phosphatase (AP) for use in methods for preserving or improving kidney in populations of SA-AKI patients, that have been determined to respond particularly well to treatment with AP.

[0050] The STOP-AKI study has identified statistically significant correlations between thresholds corresponding to degrees of severity of kidney injury and improvements in kidney function (as evidenced by improved creatinine clearance with respect to placebo) and long term survival. As discussed above, stratifying patients in the STOP-AKI clinical trial according to, e.g., markers of kidney function (e.g., ECC or eGFR) has identified particular effects of AP administration on specific subgroups. The parameter or parameters defining each of these subgroups (e.g., a series of thresholds, such as ECC or eGFR thresholds) can be used, e.g., to personalize AP therapy to specific subgroups, to select patients for treatment, to make decisions related to the AP treatment (e.g., modify dosing or dosage schedule), or to evaluate the likelihood of a positive outcome.

[0051] Accordingly, the AP for use in methods disclosed herein relate to the administration of AP, e.g., RecAP, to a subject determined to have either moderate to severe kidney injury, or with severe kidney injury, due to SA-AKI, comprising administering an AP such as RecAP to the subject. Administration of AP is particularly effective when administered to subjects having moderate to severe kidney injury or severe kidney injury at doses of at least 500 U/kg. AP was most effective when administered at doses of at least 1,000 U/kg.

[0052] As used herein, the term "severe kidney injury" refers to kidney damage or impairment of kidney function resulting in a creatinine clearance rate lower than 15 ml/min. As used herein, the term "moderate to severe kidney injury" refers to kidney damage or impairment of kidney function resulting in a creatinine clearance rate of 15 ml/min to 60 ml/min. As used herein, the term "mild kidney injury" refers to kidney damage or impairment of kidney function resulting in a creatinine clearance rate of higher than 60 ml/min. As used herein the term "baseline ECC" refers to the endogenous creatinine clearance rate at baseline. As used herein the term "baseline eGFR" refers to the estimated glomerular filtration rate at baseline. Both ECC and eGFR are estimations of the actual glomerular filtration rate as measurement of kidney function.

[0053] The present disclosure provides alkaline phosphatase (AP) for use in a method to treat sepsis-associated acute kidney disease injury (SA-AKI) in a subject in need thereof, wherein the method comprises administering an effective amount of alkaline phosphatase (AP) to said subject, wherein

1. (i) the subject has an eGFR $\leq$ 60 ml/min prior to the treatment with AP (e.g., RecAP);
2. (ii) the AP is administered in at least one dose of about 500 U/kg to about 2,000 U/kg; and,
3. (iii) the administration of the AP results in an increase in renal function.

[0054] In some aspects the subject with an eGFR $\leq$ 60 ml/min prior to the treatment with AP has 15-60 ml/min baseline eGFR. In some aspects, the subject with an eGFR $\leq$ 60 ml/min prior to the treatment with AP has <15 ml/min baseline eGFR. In some aspects, the subject with an eGFR $\leq$ 60 ml/min prior to the treatment with AP does not have >60 ml/min baseline eGFR.

[0055] The AP (e.g., RecAP) is administered as doses of at least about 500 U/kg, at least about 600 U/kg, at least about 700 U/kg, at least about 800 U/kg, at least about 900 U/kg, at least about 1000 U/kg, at least about 1100 U/kg, at least about 1200 U/kg, at least about 1300 U/kg, at least about 1400 U/kg, at least about 1500 U/kg, at least about 1600 U/kg, at least about 1700 U/kg, at least about 1800 U/kg, at least about 1900 U/kg, or at least about 2000 U/kg per dose. In some aspects, the AP (e.g. RecAP) is administered as doses above 2000 U/kg per dose. In some aspects, the AP (e.g., RecAP) is administered as doses below 500 U/kg per dose.

[0056] In some aspects, the AP (e.g., RecAP) is administered at a dose between about 500 U/kg and about 1500 U/kg, between about 600 U/kg and about 1400 U/kg, between about 700 U/kg and about 1300 U/kg, between about 800 U/kg and about 1200 U/kg, or between about 900 U/kg and about 1100 U/kg. In some specific aspects, AP is administered as about 1000 U/kg doses.

[0057] In some aspects, the AP is a human AP. In some aspects, the AP is a recombinant AP. In some aspects, the AP is a chimeric AP. In a particular aspects, the chimeric AP is RecAP (SEQ ID NO: 1). In some aspects, an AP disclosed herein has at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity to the amino acid sequence of SEQ ID NO:1. In some aspects, the AP is a functional fragment (i.e., a fragment of the AP, e.g., AP conserving at least about 10%, at least about 20%, at least about 30%, at least 40%, at least about 50%, at least about 60%, at least 70%, at least about 80%, or at least about 90% of the AP activity of the corresponding full length AP). In some aspects, the AP is a variant or a derivative of an AP disclosed herein. Other AP that can be used are discussed in detail below.

[0058] In some aspects, the AP is RecAP (e.g., the clinical grade RecAP used in the present disclosure), and it is administered at a dose of at least about 0.1 mg/kg, at least about 0.2 mg/kg, at least about 0.3 mg/kg, at least about 0.4 mg/kg, at least about 0.5 mg/kg, at least about 0.6 mg/kg, at least about 0.7 mg/kg, at least about 0.8 mg/kg, at least about 0.9 mg/kg, at least about 1 mg/kg, at least about 1.1 mg/kg, at least about 1.3 mg/kg, at least about 1.4 mg/kg, at least about 1.5 mg/kg, at least about 1.6 mg/kg, at least about 1.7 mg/kg, at least about 1.8 mg/kg, at least about 1.9 mg/kg, at least about 2 mg/kg, at least about 2.1 mg/kg, at least about 2.2 mg/kg, at least about 2.3 mg/kg, or at least about 2.4/kg per dose. In some aspects, the AP is administered as doses above 2.4 mg/kg per dose. In some aspects, the AP is RecAP (e.g., the clinical grade RecAP used in the present disclosure), and it is administered at a dose of at least about 100 U/kg, at least about 200 U/kg, at least about 300 U/kg, at least about 400 U/kg, at least about 500 U/kg, at least about 600 U/kg, at least about 700 U/kg, at least about 800 U/kg, at least about 900 U/kg, at least about 1000 U/kg, at least about 1100 U/kg, at least about 1200 U/kg, at least about 1300 U/kg, at least about 1400 U/kg, at least about 1500 U/kg, at least about 1600 U/kg, at least about 1700 U/kg, at least about 1800 U/kg, at least about 1900 U/kg, or at least about 2000 U/kg.

[0059] In some aspects, the AP is RecAP (e.g., the clinical grade RecAP used in the present disclosure) and it is administered at a dose between about 0.8 mg/kg and about 2.4 mg/kg, between about 0.9 mg/kg and about 2.3 mg/kg, between about 1 mg/kg and about 2.2 mg/kg, between about 1.1 mg/kg and about 2.1 mg/kg, between about 1.2 mg/kg and about 2 mg/kg, between about 1.3 mg/kg and about 1.9 mg/kg, between about 1.4 mg/kg and about 1.8 mg/kg, or between about 1.5 mg/kg and about 1.7 mg/kg. In some specific aspects, AP is administered as about 1.6 mg/kg doses.

[0060] In some aspects, the AP is RecAP (e.g., the clinical grade RecAP used in the present disclosure) and it has a specific activity of at least about 100 U/mg, at least about 200 U/mg, at least about 300 U/mg, at least about 400 U/mg, at least about 500 U /mg, at least about 600 U/mg, at least about 700 U/mg, at least about 800 U/mg, at least about 900 U/mg, at least about 1000 U/mg, at least about 1100 U/mg, at least about 1200 U/mg, at least about 1300 U/mg, at least about 1400 U/mg, at least about 1500 U/mg, at least about 1600 U/mg, at least about 1700 U/mg, at least about 1800 U/mg, at least about 1900 U/mg, or at least about 2000 U/mg.

[0061] In some aspects, the AP is RecAP (e.g., the clinical grade RecAP used in the present disclosure) and it has a specific activity of about 1000 U:1.6 mg. In some aspects, the AP is RecAP and it has a specific activity between about 600 U/mg and about 700 U/mg, or between about 500 U/mg and about 800 U/mg, or between about 400 U/mg and about 900 U/mg, or between about 300 U/mg and about 1000 U/mg, or between about 200 U/mg and about 1100 U/mg, or between 100 U/mg and about 1200 U/mg. In some aspects, the AP is RecAP and it has a specific activity below 100 U/mg. In some aspects, the AP is RecAP and it has a specific activity above 1200 U/mg.

[0062] In some aspects, only one dose of AP (e.g., RecAP) is administered per treatment (e.g., one dose per day for 1-7 days). In other aspects, more than one dose of AP is administered. In some aspects, two, three, four, five, six, seven, eight, nine, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or 21 doses of AP are administered (e.g., at least two doses per day for 1-7 days).

[0063] In some aspects, the AP doses are administered daily. In other aspects, AP doses are administered every 2, 3, 4, 5, 6 or 7 days.

[0064] In some aspects, a single dose is administered every day. In some aspects, 2, 3, or more doses are administered every day.

[0065] In some aspects, the treatment with AP is less than about 4 days. In some aspects, the treatment with AP is less than 3 days, less than 2 days, or less than 1 day.

[0066] In a particular aspect, the AP is administered as a daily about 1000 U/kg dose administered in 3 consecutive days. In some particular aspects, when the AP is RecAP, the AP is administered as daily 1.6 mg/kg doses administered in 3 consecutive days. In some aspects, each AP, e.g., RecAP (e.g., the clinical grade RecAP used in the present disclosure), dose is

between about 0.10 mg/kg and about 3 mg/kg, or between about 0.20 mg/kg and about 2.9 mg/kg, or between about 0.3mg/kg and about 2.8 mg/kg, or between about 0.4 mg/kg and about 2.7 mg/kg, or between about 0.5 mg/kg and about 2.6 mg/kg, or between about 0.6 mg/kg and about 2.5 mg/kg, or between about 0.7 mg/kg and about 2.4 mg/kg, or between about 0.8 mg/kg and about 2.3 mg/kg, or between about 0.9 mg/kg and about 2.2 mg/kg, or between about 1 mg and about 2.1 mg/kg, or between about 1.1 mg/kg and about 2 mg/kg, or between about 1.2 mg/kg and about 1.9 mg/kg, or between about 1.3 mg/kg and about 1.8 mg/kg, or between about 1.4 mg/kg and about 1.7 mg/kg. In some aspects, each AP, e.g., RecAP, dose comprises at least about 0.1 mg AP/kg, at least about 0.2 mg AP/kg, at least about 0.3 mg AP/kg, at least about 0.4 mg AP/kg, at least about 0.5 mg AP/kg, at least about 0.6 mg AP/kg, at least about 0.7 mg AP/kg, at least about 0.8 mg AP/kg, at least about 0.9 mg AP/kg, at least about 1 mg AP/kg, at least about 1.1 mg AP/kg, at least about 1.2 mg AP/kg, at least about 1.3 mg AP/kg, at least about 1.4 mg AP/kg, at least about 1.5 mg AP/kg, at least about 1.6 mg AP/kg, at least about 1.7 mg AP/kg, at least about 1.8 mg AP/kg, at least about 1.9 mg AP/kg, at least about 2 mg AP/kg, at least about 2.1 mg AP/kg, at least about 2.2 mg AP/kg, at least about 2.3 mg AP/kg, at least about 2.4 mg AP/kg, at least about 2.5 mg AP/kg, at least about 2.6 mg AP/kg, at least about 2.6 mg AP/kg, at least about 2.7 mg AP/kg, at least about 2.8 mg AP/kg, at least about 2.9 mg AP/kg, or at least about 3 mg AP/kg.

[0067] The AP may be administered via different routes, for example intravenously, rectally, bronchially or orally. In some specific aspects, the AP is administered intravenously, e.g., via intravenous infusion. In some aspects, AP is administered intravenously via continuous infusion.

[0068] Although short term preservation of renal function can have immediate life-saving consequences, it is preferred that the effect of AP on the renal function is long lasting.

[0069] ECC can be determined by methods known in the art. One method of calculation of ECC is to collect a urine sample (usually collected for 24-hours) to determine the amount of creatinine that was removed from the blood over a given time interval. If one removes, for instance, 1440 mg in 24 hours, this is equivalent to removing 1 mg/min. If the blood concentration is 0.01 mg/mL (1 mg/dL), then the creatinine clearance is said to be 100 mL/min. This is because, in order to excrete 1 mg of creatinine, 100 mL of blood containing 0.01 mg/mL would need to have been cleared.

[0070] eGFR can be determined by methods known in the art. Either the Modification of Diet in Renal Disease (MDRD) Study equation or the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation can be used, preferably the CKD-EPI equation. The skilled person is aware how to estimate eGFR according to either method, for instance by using a website for calculation of the eGFR as provided, for instance, by the national kidney foundation (kidney.org).

[0071] In some aspects, the administration of AP to the subject according to the methods disclosed herein is capable of improving many renal function related parameters, such as BUN,

ECC, eGFR, and serum creatinine levels in the subject.

[0072] In some aspects, the administration of AP (e.g., RecAP) to the subject results in an increase in renal function comprising an increase in ECC or eGFR with respect to ECC or eGFR, respectively, in the absence of treatment. In some aspects, the increase in ECC or eGFR is at least 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40 ml/min with respect to ECC or eGFR in the absence of treatment.

[0073] In some aspects, the administration of AP (e.g., RecAP) to the subject results in an increase in renal function comprising an increase in ECC or eGFR with respect to baseline ECC or eGFR, respectively. In some aspects, the increase in ECC or eGFR is at least 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, or 80 ml/min with respect to baseline ECC or eGFR, respectively.

[0074] In some aspects, the increase in renal function is observed 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, or 28 days after AP administration.

[0075] ECC can be used as an approximation of glomerular filtration rate (GFR). However, other parameters can also be assessed in order to determine renal function, e.g., GFR, eGFR, serum creatinine levels, electrolyte derangement, amount of produced urine, BUN, calcium levels, phosphorous levels, albumin levels, and/or red and white blood cells in urine. Another test that can be performed to determine renal function is, e.g., a complete blood count with differential. It is also possible to analyze a urine sample on the presence or absence of an RNA molecule. RNA in urine can be obtained from urine-secreted renal cells. In some aspects, the RNA is an mRNA molecule such as iNOS mRNA.

[0076] In some aspects, the administration of AP (e.g., RecAP) to the subject results in an increase of the survival rate of at least 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, or 40% compared to the survival rate in the absence of AP treatment.

[0077] Conversely, the administration of AP (e.g., RecAP) to the subject results in a decrease of the mortality rate of at least 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, or 40% compared to the mortality rate in the absence of AP treatment.

[0078] In some aspects disclosed herein, AP (e.g., RecAP) is administered to the subject only if sepsis has been detected less than 96 hours prior to the decision to initiate the treatment. In other aspects, AP is administered only if sepsis was detected less than 72 hours prior to SA-AKI detection.

[0079] In some aspects of the present disclosure, treatment with AP is initiated within 24 hours after sepsis is detected. The presence of sepsis can be detected, e.g., as disclosed in the Examples section of this application. In some aspects of the present disclosure, treatment with AP is administered to a subject at risk of sepsis in order to prevent reduction in kidney function.

[0080] In some aspects, treatment is initiated within 24 hours after SA-AKI is detected.

[0081] In some aspects disclosed herein, the administration of at least one dose of AP results in a shortening of duration or cessation of renal replacement therapy (RRT) in a subject undergoing RRT.

[0082] In some aspects disclosed herein, the administration of at least one dose of AP results in the preservation or increase of glomerular filtration rate (GFR) in the subject. GFR can be assessed in several ways, e.g., by inulin or chromium EDTA clearance, or an approximation of GFR can be made, for instance by calculating ECC. This is calculated from a measured 24 hour urine volume, the urine creatinine level, and the serum creatinine level. The GFR can also be estimated (eGFR), based on serum creatinine.

[0083] In some aspects disclosed herein, the administration of AP results in an increase of renal function or prevents the reduction of renal function below a critical threshold which would preclude the administration of a certain treatment, e.g., the administration of an antibiotic to treat sepsis. Accordingly, in some aspect, AP administration is able to prevent reduction of the renal function below a critical threshold and thus enables such person to receive the treatment. Thus, in some aspects, an indicator of renal function (e.g., ECC or eGFR) is determined prior to administering AP for preserving renal function in order to determine the risk that the renal function of said person is reduced below a certain threshold level.

[0084] The prediction of an increased clinical response to therapy with AP, e.g., RecAP, is based on baseline eGFR. The present disclosure comprises evaluating whether baseline eGFR falls within a certain range, or it's above or below a certain threshold (e.g., eGFR threshold for severity of kidney injury). Thus, if baseline eGFR, alone or in combination with other biomarkers, indicates that the patient will benefit from therapy with AP, then therapy could commence, or be maintained, or be modified (e.g., increasing or decreasing dosage, or increasing or decreasing frequency of doses).

[0085] Conversely, if baseline eGFR, alone or in combination with other biomarkers, indicates that the patient will not benefit from therapy with AP, then therapy could be discontinued, temporarily suspended, modified (e.g., increasing or decreasing dosage or increasing or decreasing frequency of doses), etc.

[0086] In other words, specific levels of baseline eGFR alone or in combination with other molecular or clinical biomarkers are correlated with clinical efficacy of AP therapy and useful to predict clinical outcomes in specific populations of patients suffering from SA-AKI.

[0087] The invention thus provides alkaline phosphatase (AP) for use in a method to treat sepsis-associated acute kidney disease injury (SA-AKI) comprising administering an effective amount of alkaline phosphatase (AP) to said subject, wherein

1. (i) the subject has an eGFR (estimated glomerular filtration rate) ≤ 60 ml/min prior to the treatment with AP, and
2. (ii) the AP is to be administered in at least one 500 U/kg to 2,000 U/kg dose. In a preferred embodiment, the subject has 15-60 ml/min eGFR prior to the treatment with AP. In another preferred embodiment, the subject has <15 ml/min eGFR prior to the treatment with AP.

[0088] In one preferred embodiment, an AP for use according to the invention is provided, wherein the AP is a human AP.

[0089] In a preferred embodiment, the AP is a recombinant AP, preferably chimeric, more preferably having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 100% sequence identity to the amino acid sequence of RecAP (SEQ ID NO: 1).

[0090] In a preferred embodiment, an AP for use according to the invention is provided, wherein the administration of AP leads to an increase in renal function and wherein the increase in renal function comprises an increase in ECC with respect to ECC in the absence of treatment or an increase in eGFR with respect to eGFR in the absence of treatment.

[0091] In one preferred embodiment, an AP for use according to the invention is provided, wherein sepsis is detected less than 96 hours prior to AP administration.

[0092] In one preferred embodiment, an AP for use according to the invention is provided, wherein the sepsis is detected less than 72 hours prior to SA-AKI detection.

[0093] Preferably, treatment is initiated within 24 hours after sepsis is detected and/or after SA-AKI is detected.

[0094] In a preferred embodiment, AP for use according to the invention is provided, wherein AP is administered once daily. In a preferred embodiment, AP is administered intravenously. In a preferred embodiment, AP is administered in three daily doses.

[0095] In a preferred embodiment, AP for use according to the invention is provided, wherein the AP is RecAP and the dose is between 0.06 mg/kg (and/or 375 U/kg) or 3.2 mg/kg (and/or 2,000 U/kg) of RecAP, preferably between 0.08 mg/kg (and/or 500 U/kg).

[0096] In one preferred embodiment, AP for use according to the invention is provided,

wherein the administration of at least one dose of AP results in a shortening of duration or cessation of renal replacement therapy (RRT) in a subject undergoing RRT.

[0097] In one preferred embodiment, AP for use according to the invention is provided, wherein the administration of at least one dose of AP results in the preservation or increase of glomerular filtration rate (GFR) in the subject.

[0098] The term "biomarker" as used herein refers to a factor that is a distinctive indicator of a biological process, biological event, and/or pathologic condition, e.g., a predictor of clinical response to treatment with AP, e.g., RecAP. As used herein, the term biomarker encompasses both clinical markers and molecular biomarkers (biological markers). Thus, in the context of the present disclosure, the term "biomarker" encompasses, e.g., "biological biomarkers" or "molecular biomarkers." In some aspects, the biological or molecular biomarkers used to evaluate kidney function comprise markers of liver function (e.g., alanine aminotransferase, aspartate aminotransferase, gamma-glutamyl transferase, bilirubin, or LDH), C-reactive protein (CRP), interleukin 6 (IL-6), interleukin 18 (IL-18), lipopolysaccharide binding protein, kidney injury molecule 1 (KIM-1) or combinations thereof.

[0099] As disclosed above, the term "biomarker" also encompasses "clinical biomarkers," also referred to as "clinical status markers," that can be predictive of response to biological therapies, for example, gender, age, concomitant drugs, smoking status, body mass index (BMI), etc.

[0100] As discussed above, eGFR is used for a cut-off approach. Thus, if a subject's eGFR at baseline is below 60 ml/min that subject would become a candidate for treatment with a certain AP therapy, e.g., therapy with a certain AP regimen comprising one or more doses of RecAP.

[0101] In some aspects, the mere determination that the eGFR is below a predetermined threshold level would suffice to identify a subject as a candidate for treatment with a certain AP therapy, e.g., therapy with RecAP. Thus, in some aspects of the methods disclosed herein, eGFR can be used alone. However, in other aspects, the eGFR can be combined with each other or with other measures of kidney function (e.g., BUN clearance) and/or molecular or clinical biomarkers, such KIM-1 levels.

[0102] These findings can be applied, for example, to devising new methods of determining treatment (e.g., by selecting patients as candidates for a certain AP therapy), methods of treating a decrease in kidney function, preventing a decrease in kidney function, increasing kidney function, or preserving kidney function (e.g., to treat SA-AKI), methods of monitoring efficacy of an AP treatment, or methods to adjust formulations, dosage regimens, or routes of administration.

[0103] The AP for use in methods disclosed herein include prescribing, initiating, and/or altering prophylaxis and/or treatment for SA-AKI, based at least in part on a subject's eGFR, alone or in combination with one or more additional biomarkers.

[0104] The present disclosure not forming a part of the invention provides a method of determining whether to treat a patient having SA-AKI with a therapeutic regimen comprising the administration of an AP wherein the method comprises: (a) measuring or instructing a clinical laboratory to measure eGFR, and optionally levels of additional biomarkers such as KIM-1 in a sample taken from the patient, and (b) treating or instructing a healthcare provider to treat the patient, or suspending the treatment, not initiating the treatment, denying the treatment, or instructing a healthcare provider to suspend, not initiate, or deny the treatment with a therapeutic regimen comprising the administration of an AP, e.g., RecAP, if the patient is determined to have higher or lower eGFR, and optionally levels of additional biomarkers such as KIM-1 in the sample compared to each biomarker predetermined threshold level or levels, or compared to each biomarker level or levels in one or more controls. Also provided is a method of determining whether a patient having SA-AKI is likely to respond to a therapeutic regimen comprising the administration of an AP, wherein the method comprises: (a) measuring or instructing a clinical laboratory to measure eGFR, and optionally levels of additional biomarkers such as KIM-1 in a sample taken from the patient, and (b) determining that the patient is likely to respond, or is not likely to respond, to the treatment with a therapeutic regimen comprising the administration of an AP, e.g., RecAP, if the patient is determined to have higher or lower eGFR, and optionally levels of additional biomarkers such as KIM-1 in the sample compared to each biomarker predetermined threshold level or levels, or compared to each biomarker level or levels in one or more controls.

[0105] In one aspect, the disclosure not forming a part of the invention provides a method of determining whether to treat a patient having SA-AKI with a therapeutic regimen comprising the administration of an AP wherein the method comprises: (a) measuring or instructing a clinical laboratory to measure eGFR and optionally levels of additional biomarkers such as KIM-1 in a sample taken from the patient, and (b) treating or instructing a healthcare provider to treat the patient with a therapeutic regimen comprising the administration of an AP if the patient is determined to have lower or decreased eGFR, and higher or increased levels of at least one optional additional biomarker such as KIM-1 in the sample compared to a predetermined biomarker threshold level or levels, or compared to a biomarker level or levels in one or more controls. Also provided is a method of determining whether a patient having SA-AKI is likely to respond to a therapeutic regimen comprising the administration of an AP, wherein the method comprises: (a) measuring or instructing a clinical laboratory to measure eGFR and optionally levels of additional biomarkers such as KIM-1 in a sample taken from the patient, and (b) determining that the patient is likely to respond to a therapeutic regimen comprising the administration of an AP if the patient is determined to have lower or decreased eGFR, and higher or increased levels of at least one optional additional biomarker such as KIM-1 in the sample compared to a predetermined biomarker threshold level or levels, or compared to a biomarker level or levels in one or more controls.

[0106] In one aspect, the disclosure not forming a part of the invention provides a method of determining whether to treat a patient having SA-AKI with a therapeutic regimen comprising the administration of an AP wherein the method comprises (a) measuring or instructing a

clinical laboratory to measure the eGFR and optionally levels of additional biomarkers such KIM-1 in a sample taken from the patient, and (b) suspending the treatment, not initiating treatment, denying the treatment, or instructing a healthcare provider to suspend, not initiate, or deny the treatment of the patient with a therapeutic regimen comprising the administration of an AP, e.g., RecAP, to the patient if the patient is determined to have higher or increased eGFR, and lower or decreased levels of at least one optional additional biomarker such as KIM-1 in the sample compared to a predetermined biomarker threshold level or levels, or compared to a biomarker level or levels in one or more controls. Also provided is a method of determining whether to treat a patient having SA-AKI with a therapeutic regimen comprising the administration of an AP wherein the method comprises (a) measuring or instructing a clinical laboratory to measure the eGFR and optionally levels of additional biomarkers such KIM-1 in a sample taken from the patient, and (b) determining that the patient is unlikely to respond to a therapeutic regimen comprising the administration of an AP, e.g., RecAP, to the patient if the patient is determined to have higher or increased eGFR, and lower or decreased levels of at least one optional additional biomarker such as KIM-1 in the sample compared to a predetermined biomarker threshold level or levels, or compared to a biomarker level or levels in one or more controls.

[0107] Also provided but not forming a part of the invention is a method of selecting a patient diagnosed with SA-AKI as a candidate for treatment with an AP, comprising (a) measuring or instructing a clinical laboratory to measure eGFR and optionally levels of additional biomarkers such as KIM-1 in a sample taken from the patient, and (b) treating or instructing a healthcare provider to treat the patient with an AP if the patient is determined to have lower or decreased eGFR, and higher or increased levels of at least one optional additional biomarker such as KIM-1 in the sample compared to a predetermined threshold level or levels, or compared to a biomarker level or levels in one or more controls. Also provided is a method of selecting a patient diagnosed with SA-AKI as a candidate for treatment with an AP, comprising (a) measuring or instructing a clinical laboratory to measure eGFR and optionally levels of additional biomarkers such as KIM-1 in a sample taken from the patient, and (b) determining that the patient is likely to respond to a treatment with AP if the patient is determined to have lower or decreased eGFR (or another kidney function parameter), and higher or increased levels of at least one optional additional biomarker such as KIM-1 in the sample compared to a predetermined threshold level or levels, or compared to a biomarker level or levels in one or more controls.

[0108] Also provided but not forming a part of the invention is a method of selecting a patient diagnosed with SA-AKI as a candidate for treatment with an AP comprising (a) measuring or instructing a clinical laboratory to measure eGFR and optionally levels of additional biomarkers such KIM-1 in a sample taken from the patient, and (b) suspending the treatment, not initiating treatment, denying the treatment, or instructing a healthcare provider to suspend, not initiate, or deny the treatment of the patient with an AP, e.g., RecAP, to the patient if the patient is determined to have higher or increased eGFR, and lower or decreased levels of at least one optional additional biomarker such as KIM-1 in the sample compared to a predetermined threshold level or levels, or compared to a biomarker level or levels in one or more controls.

Also provided is a method of selecting a patient diagnosed with SA-AKI as a candidate for treatment with an AP comprising (a) measuring or instructing a clinical laboratory to measure eGFR and optionally levels of additional biomarkers such as KIM-1 in a sample taken from the patient, and (b) determining that the patient is unlikely to respond to treatment with an AP, e.g., RecAP, if the patient is determined to have higher or increased eGFR, and lower or decreased levels of at least one optional additional biomarker such as KIM-1 in the sample compared to a predetermined threshold level or levels, or compared to a biomarker level or levels in one or more controls.

[0109] In some aspects, the AP for use in methods can entail ordering and/or performing one or more additional assays. For example, the eGFR determination assay may be repeated to rule out a false negative result, and/or one or more additional eGFR determination assays may be performed to monitor the subject's status. Conversely, it may be desirable repeat the eGFR determination assays to rule out a false positive result.

[0110] The predetermined ECC or eGFR threshold level is about 15 ml/min (threshold differentiating between severe kidney injury and moderate to severe kidney injury), or about 60 ml/min (threshold differentiating between moderate to severe kidney injury and mild kidney injury).

[0111] The presence of eGFR above or below a predetermined threshold level in a patient with SA-AKI can be used in combination with one or more of clinical or molecular biomarkers specific for sepsis, or for the specific infection that lead to sepsis.

[0112] A person skilled in the art would understand that eGFR can be used according to the methods disclosed herein, including but not limited to treatment, diagnostic, and monitoring methods, as a positive selector, *i.e.*, a specific action would be taken (e.g., treating a patient) if the eGFR value in a sample taken from the patient is below or above a predetermined eGFR threshold level, or eGFR is increased or decreased relative to the eGFR in one or more controls.

[0113] A person skilled in the art would understand that eGFR can be used according to the methods disclosed herein, including but not limited to treatment, diagnostic, and monitoring methods, as a negative selector, *i.e.*, a specific action would not be taken (e.g., treating a patient) if eGFR value in a sample taken from the patient is below or above a predetermined eGFR threshold level, or eGFR is increased or decreased relative to the eGFR in one or more controls.

[0114] The disclosure includes methods to facilitate a determination by a healthcare provider, a healthcare benefits provider, or a clinical laboratory to as to whether a patient will benefit from treatment with an AP.

[0115] A diagnosis may be a differential diagnosis, based at least in part on eGFR of a patient. In some aspects, the methods disclosed herein include informing the subject of a result of the

eGFR determination assay and/or of a diagnosis based at least in part on eGFR. The patient can be informed verbally, in writing, and/or electronically. This diagnosis can also be recorded in a patient medical record.

[0116] The term "medical record" or "patient medical record" refers to an account of a patient's examination and/or treatment that typically includes one or more of the following: the patient's medical history and complaints, the physician's physical findings, the results of diagnostic tests and procedures, and patient medications and therapeutic procedures. A medical record is typically made by one or more physicians and/or physicians' assistants and it is a written, transcribed or otherwise recorded record and/or history of various illnesses or injuries requiring medical care, and/or inoculations, and/or allergies, and/or treatments, and/or prognosis, and/or frequently health information about parents, siblings, and/or occupation. The record may be reviewed by a physician in diagnosing the condition.

[0117] The medical record can be in paper form and/or can be maintained in a computer-readable medium. The medical record can be maintained by a laboratory, physician's office, a hospital, a healthcare maintenance organization, an insurance company, and/or a personal medical record website. In some aspects, a diagnosis, based at least in part on the measured eGFR, is recorded on or in a medical alert article such as a card, a worn article, and/or a radiofrequency identification (RFID) tag. As used herein, the term "worn article" refers to any article that can be worn on a subject's body, including, but not limited to, a tag, bracelet, necklace, arm band, or head band.

[0118] As used herein, the term "diagnosis" means detecting a disease or determining the stage or degree of a disease. Usually, a diagnosis of a disease is based on the evaluation of one or more factors and/or symptoms that are indicative of the disease. That is, a diagnosis can be made based on the presence, absence or amount of a factor which is indicative of presence or absence of the disease or disorder. Each factor or symptom that is considered to be indicative for the diagnosis of a particular disease does not need be exclusively related to the particular disease, e.g. there may be differential diagnoses that can be inferred from a diagnostic factor or symptom. Likewise, there may be instances where a factor or symptom that is indicative of a particular disease is present in an individual that does not have the particular disease.

[0119] The term "diagnosis" also encompasses determining the therapeutic effect of a drug therapy, e.g., AP therapy, or predicting the pattern of response to a drug therapy. The diagnostic methods may be used independently, or in combination with other diagnosing and/or staging methods known in the medical arts for a particular disease.

[0120] As used herein, the term "differential diagnosis" refers to the determination of which of two or more diseases with similar symptoms is likely responsible for a subject's symptom(s), based on an analysis of the clinical data. The term is also used to refer to the determination of whether a patient is susceptible to treatment with an AP depending on whether the measured eGFR in a patient sample is above or below a predetermined threshold level, or elevated or

decreased relative to the level in one or more controls.

[0121] The term "prognosis" as used herein refers to a prediction of the probable course and outcome of a clinical condition or disease, e.g., sepsis or SA-AKI. A prognosis is usually made by evaluating factors or symptoms of a disease that are indicative of a favorable or unfavorable course or outcome of the disease. The phrase "determining the prognosis" as used herein refers to the process by which the skilled artisan can predict the course or outcome of a condition in a patient. The term "prognosis" does not refer to the ability to predict the course or outcome of a condition with 100% accuracy. Instead, the skilled artisan will understand that the term "prognosis" refers to an increased probability that a certain course or outcome will occur; that is, that a course or outcome is more likely to occur in a patient exhibiting a given condition, when compared to those individuals not exhibiting the condition.

[0122] The terms "favorable prognosis" and "positive prognosis," or "unfavorable prognosis" and "negative prognosis" as used herein are relative terms for the prediction of the probable course and/or likely outcome of SA-AKI. A favorable or positive prognosis predicts a better outcome for a condition than an unfavorable or negative prognosis. In a general sense, a "favorable prognosis" is an outcome that is relatively better than many other possible prognoses that could be associated with a particular condition, whereas an unfavorable prognosis predicts an outcome that is relatively worse than many other possible prognoses that could be associated with a particular condition. Typical examples of a favorable or positive prognosis include increased kidney function, preservation of kidney function, increase in ECC or eGFR (or another kidney function parameter), and the like.

[0123] The disclosure provides AP for use in a method of treating SA-AKI in a subject, wherein the method comprises: administering an AP to the patient if the patient is determined to have a lower or decreased eGFR in one or more samples taken from the patient compared to predetermined eGFR.

[0124] The disclosure also provides a AP for use in a method of treating a patient having SA-AKI, wherein the method comprises: (a) submitting a sample taken from the patient for measurement of eGFR in the sample, and (b) administering an AP to the patient if the patient has a lower or decreased eGFR in the sample taken compared to a predetermined eGFR threshold level.

[0125] Also provided is AP for use in a method of treating a patient having SA-AKI, wherein the method comprises: (a) submitting a sample taken from the patient for measurement of eGFR in the sample, and (b) suspending or not initiating the administration of AP, e.g., RecAP, to the patient if the patients has a higher or increased eGFR in the sample compared to a predetermined eGFR threshold level.

[0126] The disclosure also provides AP for use in a method of treating a patient having SA-AKI, wherein the method comprises: (a) measuring eGFR in a sample obtained from the patient; and (b) determining whether eGFR values in the sample is higher or increased, or

lower or decreased compared to a predetermined eGFR threshold level. In some aspects the method further comprises administering or advising a healthcare provider to administer an AP, e.g., RecAP, to the patient if the patient is determined to have a lower or decreased eGFR in the sample compared to a predetermined eGFR threshold level; or to suspend or deny the administration of an AP if the patient is determined to have a higher or increased eGFR level in the sample compared to a predetermined eGFR threshold level.

[0127] Also provided is AP for use in a method of treating a patient having SA-AKI, wherein the method comprises: (a) submitting a sample taken from a patient for measurement of eGFR in a sample obtained from the patient, and (b) administering an AP to the patient if the patient is determined to have a lower or decreased eGFR measured from the sample compared to a predetermined eGFR threshold level; or suspending, not initiating, or denying the administration of an AP to the patient if the patient is determined to have a higher or increased eGFR in the sample compared to a predetermined eGFR threshold level.

[0128] In some aspects, the sample is a urine sample obtained from the patient and is submitted for estimation of eGFR in the sample, for example, to a clinical laboratory.

[0129] In some aspects, the eGFR determination assay is performed on a sample obtained from the patient, by the healthcare professional treating the patient. In some aspects, a sample is obtained from the patient and is submitted, e.g., to a clinical laboratory, for measurement of eGFR in the sample according to the healthcare professional's instructions. In some aspects, the clinical laboratory performing the assay will advise the healthcare provider as to whether the patient can benefit from treatment with an AP based on whether the patient's eGFR is above or below a predetermined eGFR threshold value.

[0130] The disclosure not forming a part of the invention also relates to a method of measuring the efficacy or pharmacodynamics of an AP in a patient diagnosed with SA-AKI, comprising: (a) conducting a first measurement of the patients' eGFR in a first sample taken from the patient; (b) administering the AP, e.g., RecAP; and (c) conducting a second measurement of eGFR in a second sample taken from the patient, wherein an increase of the eGFR in the second measurement compared to the patient's eGFR in the first measurement, indicates that the patient is responding to treatment with the AP, e.g., RecAP. The disclosure also relates to a method of measuring the efficacy or pharmacodynamics of an AP in a patient diagnosed with SA-AKI, comprising: (a) conducting a first measurement of the patients' eGFR in a first sample taken from the patient; and (b) conducting a second measurement of eGFR in a second sample taken from the patient after the patient has been provided with the AP, e.g. RecAP, wherein an increase of the eGFR in the second measurement compared to the patient's eGFR in the first measurement, indicates that the patient is responding to treatment with the AP, e.g., RecAP.

[0131] The disclosure not forming a part of the invention also relates to a method of measuring the efficacy or pharmacodynamics of an AP in a patient diagnosed with SA-AKI, comprising: (a) conducting a first measurement of the patients' eGFR in a first sample taken from the patient;

(b) administering the AP, e.g., RecAP; and (c) conducting a second measurement of eGFR in a second sample taken from the patient, wherein a decrease of the eGFR in the second measurement compared to the patient's eGFR in the first measurement, indicates that the patient is not responding to treatment with the AP, e.g., RecAP. The disclosure also relates to a method of measuring the efficacy or pharmacodynamics of an AP in a patient diagnosed with SA-AKI, comprising: (a) conducting a first measurement of the patients' eGFR in a first sample taken from the patient; and (b) conducting a second measurement of eGFR in a second sample taken from the patient after the patient has been provided with the AP, e.g. RecAP, wherein a decrease of the eGFR in the second measurement compared to the patient's eGFR in the first measurement, indicates that the patient is not responding to treatment with the AP, e.g., RecAP.

[0132] In some aspects, the second measurement is conducted 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, or 28 days, or at 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or 16 weeks, or at intervening times, after administering the AP, e.g., RecAP.

[0133] In certain aspects, a "loading" dose of an AP is administered to achieve a desired level of kidney function in the patient. If the AP loading dose does not affect the patient's kidney function significantly a decision could be made to discontinue treatment - e.g., to switch to an alternative therapy.

[0134] If the loading dose results in increased kidney function in the patient a decision could be made to reduce the AP dose size or frequency to a "maintenance" dose. It is important to note that the methods provided here are guidelines for a healthcare provider to administer treatment, and the ultimate treatment decision will be based on the healthcare provider's sound judgment.

[0135] In some aspects, this disclosure includes AP for use in a method of treating a patient having SA-AKI, wherein the method comprises: measuring, e.g., in a clinical laboratory, the eGFR level and optionally one or more biomarker levels in a first sample obtained from a patient having SA-AKI, e.g., a sample provided by a healthcare provider, wherein the patient's eGFR level in the first sample is, for example, measured; determining whether the patient's eGFR level in the first sample is below a predetermined eGFR threshold level; and advising a healthcare provider to administer an AP, e.g., RecAP, to the patient if the patient's eGFR level is below a predetermined eGFR threshold level.

[0136] In some aspects, a sample is obtained from the patient (e.g., a 24 hour urine sample or a blood sample) and is submitted, e.g., to a clinical laboratory, for measurement of eGFR alone or in combination with the level of at least another biomarker; or a combination thereof in the sample.

[0137] In some aspects, the clinical laboratory performing the assay will advise the healthcare provider as to whether the patient can benefit from treatment with an AP based on whether the

patient's eGFR is below or above a predetermined eGFR threshold value.

[0138] The formulation, dosage regimen, and route of administration of an AP, e.g., RecAP, can be adjusted to provide an effective amount for an optimum therapeutic response according to the method disclosed herein. With regard to the administration of an AP, the AP may be administered through any suitable means, compositions and routes known in the art. With regard to dosage regimens, a single bolus can be administered, several divided doses can be administered over time or the dose can be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation.

III. Alkaline Phosphatases (AP)

[0139] Alkaline phosphatase (AP; EC 3.1.3.1 according to IUBMB Enzyme Nomenclature), is an enzyme that catalyzes the reaction of a phosphatase monoester and H₂O to an alcohol and phosphate. Other name(s) for AP are alkaline phosphomonoesterase; phosphomonoesterase; glycerophosphatase; alkaline phosphohydrolase; alkaline phenyl phosphatase; orthophosphoric-monoester phosphohydrolase (alkaline optimum). The systemic name of AP is phosphate-monoester phosphohydrolase (alkaline optimum).

[0140] AP is a wide specificity enzyme, it also catalyzes transphosphorylations. In humans and other mammals at least four distinct but related AP are known. They are intestinal, placental, placental-like, and liver/bone/kidney (or tissue non-specific) AP. The first three are located together on chromosome 2 while the tissue non-specific form is located on chromosome 1.

[0141] The term "AP of the present disclosure" refers to isolated alkaline phosphatases, including splice variants, isoforms, and polymorphic forms thereof. Also included are recombinant AP and chimeric AP. In a specific aspect, the AP is RecAP. The amino acid sequence of RecAP is shown in FIG. 2. In some aspects, an AP disclosed herein has at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity to the amino acid sequence of SEQ ID NO:1. In some aspects, the AP is a functional fragment (i.e., a fragment of the AP, e.g., AP conserving at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least 70%, at least about 80%, or at least about 90% of the AP activity of the corresponding full length AP). In some aspects, the AP is a variant or a derivative of an AP disclosed herein.

[0142] An AP for use according to the present disclosure can be a commercial AP enzyme, or any composition comprising the AP enzyme and any means capable of producing a functional AP enzyme in the context of the current invention, such as DNA or RNA nucleic acids encoding an AP protein.

[0143] The nucleic acid encoding AP may be embedded in suitable vectors such as plasmids, phagemids, phages, (retro)viruses, transposons, gene therapy vectors and other vectors

capable of inducing or conferring production of AP. Also native or recombinant microorganisms, such as bacteria, fungi, protozoa and yeast may be applied as a source of AP in the context of the current disclosure.

[0144] AP containing compositions for use according to the present disclosure can comprise a eukaryotic AP, e.g., a mammalian AP, which may be of the types tissue non-specific AP, such as liver-bone or kidney type, or tissue specific such as placental AP, intestinal AP and placental-like AP. The latter, also known as germ cell AP, is localized to testis, thymus and certain germ cell tumors, and is closely related to both the placental and intestinal forms of AP.

[0145] In some aspects, the mammalian AP is a human or a bovine AP. Non-limiting examples of a human AP sequence can be found in the NCBI (Genpept) collection and include: NP_001622 (intestinal AP), NP_001623 (placental AP), NP_112603 (placental-like AP) or NP_000469 (tissue non-specific AP). In some aspects, the AP comprises a polymorphism. In some aspects, the AP is placental AP, placental-like AP, intestinal AP, liver/bone/kidney AP, or a combination thereof. In some aspects, the AP is recombinant AP.

[0146] From a conformational point of view, an AP roughly consists of two domains: a crown domain and an active-site domain. The active-site domain can be divided in separate parts like the catalytic residue and the three metal ion sites (Zn1, Zn2 and Mg3). From a primary structure point of view, the crown domain is flanked by the amino acids that form the active site domain. The amino acid sequence of APs and the relative positions of the catalytic and crown domain are known by the skilled person.

[0147] In some aspects of the present disclosure, the AP is an isolated or recombinant AP comprising a crown domain and a catalytic domain, wherein said crown domain and said catalytic domain are obtained from different APs and wherein at least one of said different phosphatases is a human phosphatase. In some aspects, the AP is, for example, ECAP (*Escherichia coli* AP) or one of the seven known BIAPs (Bovine Intestinal AP).

[0148] In some aspects, AP is an isolated or recombinant AP comprising a crown domain and a catalytic domain, wherein said crown domain and said catalytic domain are obtained from different APs and wherein the different APs are human APs. This is especially useful if the modified phosphatase is subsequently used in human therapy. AP for use in the disclosed methods can be modified, e.g., genetically modified, APs of human origin which are not or only weakly immunogenic.

[0149] A modified AP disclosed herein can be used, for example, in "in vitro" or "ex vivo" diagnostics or treatments. Such modified phosphatase can comprise, for example, a human and an *E.coli* AP or may be composed of a bovine and an *E.coli* AP.

[0150] In some aspects of the present disclosure, the AP is an isolated or recombinant AP comprising a crown domain and a catalytic domain, wherein said crown domain and said catalytic domain are obtained from different APs and wherein said crown domain is the crown

domain of placental AP (ALPP) and wherein said catalytic domain is the catalytic domain of intestinal AP (ALPI). In some aspects, at least one of the different APs is a human phosphatase. In other aspects, both different APs are human phosphatases.

[0151] Domain swapped mutants suitable for the methods disclosed herein which are based on the human APs are listed in TABLE 1.

TABLE 1 Domains swapped alkaline phosphatase enzymes. ALPI is intestinal AP, ALPP is placental AP, GCAP is placental-like AP and TNAP is tissue non-specific AP.

Catalytic domain	Crown domain	Referred to as
ALPI	GCAP	catALPI/crownGCAP
	TNAP	catALPI/crownTNAP
ALPP	GCAP	catALPP/crownGCAP
	TNAP	catALPP/crownTNAP
GCAP	ALPI	catGCAP/crownALPI
	ALPP	catGCAP/crownALPP
	TNAP	catGCAP/crownTNAP
TNAP	ALPI	catTNAP/crownALPI
	ALPP	catTNAP/crownALPP
	GCAP	catTNAP/crownGCAP

[0152] In some aspects, the AP is a combination between the catalytic domain of ECAP or any of the human forms (ALPI, ALPP, GCAP or TNAP) with the crown domain of BIAP. Moreover, combinations of the crown domain of BIAP with the catalytic domain of ECAP or any of the human forms can also be produced.

[0153] In some aspects, the modified AP is an AP which under natural conditions are linked to the membrane of a cell via a glycosylphosphatidylinositol (GPI) anchor but which is modified such that it is no longer attached to the membrane of a cell. All isoenzymes are functionally active in the cell membrane and GPI-anchor deficient forms are not naturally present at detectable levels. Although serum AP activity has been demonstrated it is generally accepted that the enzyme is still present in shed membrane fractions or membrane vesicles. AP activity in milk is also present in fractions containing membrane vesicles. The GPI anchor is stored as a precursor molecule in the cell where it is attached to the attachment site through a transamidase. The backbone of the GPI-anchor is identical in mammals, but cell-type dependent modifications are known.

[0154] In some aspects, for treatment of human subjects, the AP is human. This is primarily due to the fact that AP forms obtained from other species may be immunogenic in human subjects and treatment could elicit immunological reactions and pathological side effects. In some subjects even lethal side effects, i.e., anaphylactic shock may occur and the risks of

immunological side effects are therefore preferably minimized by use of human AP forms.

[0155] As isolation of AP from humans is not practical, human recombinant forms of AP proteins can be routinely produced in different recombinant expression platforms. However, expression and purification of GPI modified and membrane-anchored proteins is notoriously difficult; GPI proteins are difficult to separate from membranes and difficult to isolate and purify. Thus, in some aspects, the recombinant APs comprises a modification in the GPI signal sequence, wherein said modification results in a secreted AP, i.e., the AP is not attached to the cell membrane.

[0156] There is no general sequence responsible for the attachment of a GPI anchor, but there some specific consensus characteristics:

1. (i) hydrophobic stretch of amino acids at the C-terminus (at least 11 amino acids, but preferably more than 11 amino acids);
2. (ii) upstream of the hydrophobic region, a spacer of hydrophilic amino acids (5-12 amino acids);
3. (iii) GPI is attached to a small amino acid: glycine, aspartic acid, asparagine, alanine, serine or cysteine; and,
4. (iv) the 2 subsequent amino acids downstream of the GPI attachment site must be small amino acids and in the majority of cases they are selected from glycine, aspartic acid, asparagine, alanine, serine or cysteine.

[0157] In some aspects, the recombinant AP comprises a modification in the GPI signal sequence, wherein said modification results in a secreted AP that is biological active, i.e., it shows activity towards a biologically relevant substrate. In some aspects, the secreted AP is a human AP. In some aspects, the secreted human AP human liver-kidney-bone phosphatase, human intestinal AP, or human placental-like alkaline phosphatase.

[0158] Based on the consensus characteristics above, a skilled person can introduce modifications, e.g., by inserting one or multiple amino acids, that would disrupt part of the consensus a result in an AP not capable to attaching a GPI anchor. Thus, in some aspects, the recombinant AP comprises a modification in the GPI signal sequence which results in a secreted AP, wherein the modification comprises a mutation or a deletion of at least one amino acid in the sequence encompassing the consensus GPI signal sequence.

[0159] In some aspects, the AP is an AP disclosed in U.S. Patent No. 8,557,545. In some aspects, the AP is a chimeric AP or chimeric AP-like protein such as those described in in U.S. Patent Appl. Publ. No. US2017/0009216 and US2014/0193388. In some specific aspects of the present disclosure, the AP is a recombinant alkaline phosphatase comprising the catalytic domain of ALPI (intestinal alkaline phosphatase) and the crown domain of ALPP (placental alkaline phosphatase), e.g., RecAP (SEQ ID NO: 1). RecAP is also known as catALPI/crownALPP, Xinlap, and sALPI-ALPP-CD. In some aspects, the AP is an improved

RecAP, e.g., LVL-RecAP (corresponding to SEQ ID NO:1 in U.S. Patent Appl. Publ. No. US2017/0009216).

[0160] In some aspects, an AP of the present disclosure comprises (i) a sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least 99% sequence identity with the crown domain of a human ALPP, and (ii) a sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least 99% sequence identity with the catalytic domain of a human ALPI.

[0161] In some aspects, said sequence having said sequence identity to the crown domain of ALPP is situated in a protein according to the invention at approximately the same position as the crown domain of ALPP in the native ALPP protein

[0162] The percentage of identity of an amino acid or nucleic acid sequence, or the term "% sequence identity", is defined herein as the percentage of residues in a candidate amino acid or nucleic acid sequence that is identical with the residues in a reference sequence after aligning the two sequences and introducing gaps, if necessary, to achieve the maximum percent identity. In a preferred embodiment, the calculation of said at least percentage of sequence identity is carried out without introducing gaps. Methods and computer programs for the alignment are well known in the art, for example "Align 2" or the BLAST service of the National Center for Biotechnology Information (NCBI).

V. Amelioration of Adverse Effects from Treatments and Diagnostics

[0163] In some aspects, the AP for use in methods disclosed herein can be applied to SA-AKI patients that have to undergo renal replacement, subjected to the use of contrast media for diagnostic purposes, etc.

[0164] *Renal Replacement Therapy:* Reduced renal function can be an indication for RRT, but in some cases reduced renal function may also be a contra-indication for RRT. This is explained by the fact that RRT generally is accompanied by intravasal fluid depletion and critically ill patients do not tolerate fluctuations in amount of intravasal fluid. This may ultimately lead to hypovolemic shock, which compromises the blood flow to the kidneys, exposing the kidneys to an ischemic insult. Complete loss of residual renal function has been observed in subjects receiving chronic RRT. The present invention provides AP for use in a method to decrease and/or prevent loss of renal function in SA-AKI patients, e.g., complete loss of residual renal function, in a subject receiving chronic RRT, wherein an AP, preferably an AP as disclosed herein, e.g., RecAP, is administered before and/or during RRT.

[0165] *Contrast media:* Another treatment with a risk of reducing renal function is the use of intravascular contrast media, such as for instance iodinated contrast media. Especially the

ionic contrast media, which are highly hyperosmolar in comparison with (blood)plasma are often associated with a reduction in renal function. The effect of ionic contrast media may be explained as follows. The hyperosmolar contrast agent may attract extravasal fluids into the vascular system, which can cause the arteries of the kidneys to expand. When the arteries expand vasoconstrictors are released to compensate for the artery expansion. These actions can result in a rapid opening and closing action of the arteries. The result of this action is a diminished blood supply to kidneys which can lead to total shut down of the kidneys. Arteries can also be constricted to an extent that they totally close. The body attempts to regulate the fluid overload in the vascular system. However, if the kidneys are non-functional, either as a result of the contrast media or independent thereof, the fluid is forced to seek other avenues of escape causing fluid overload to occur in other body systems. One of the major results of this event is pulmonary edema. Accordingly, the present disclosure provides AP for use in methods to retain or improve renal function in SA-AKI patients, when ionic contrast media are used comprising administering an AP, preferably an AP as disclosed herein, e.g., RecAP. In such case, a patient requiring contrast media can receive such necessary treatment with reduced risk of reducing renal function. In cases where contrast media was contra-indicated in SA-AKI patients with (severe) reduced renal function, due to alternative treatment that could preserve or improve renal function, it is now possible to treat such patients with an AP, preferably an AP as disclosed herein, e.g., RecAP, in order to enable them to receive such contrast media. In some aspects, an AP, preferably an AP as disclosed herein, e.g., RecAP, can be administered before and/or during and/or after administration of contrast media. As used herein, the term "contrast media" encompasses not only a single contrast medium (contrast agent) but also combinations of two or more contrast media.

VI. Reduction of Co-medication

[0166] In some aspects, AP, preferably RecAP or any of the AP disclosed above, for use in a method to treat SA-AKI can reduce the amount of (co)medication, thereby at least in part preserving and/or increasing renal function. In some aspects, the (co)medication is for the treatment of a heart condition, e.g., a heart condition resulting from myocardial infarction or heart failure. In another aspect, the (co)medication is a diuretic medicament. Diuretic medicaments influence the fluid balance in an individual by forced diuresis. Diuretics have been proven useful for, amongst others, the treatment of heart failure, hypertension and renal diseases. On the other hand, in a person already suffering from reduced renal function, diuretics may reduce renal function even further, as a result of the reduction in blood pressure due to diuresis. AP, however, is able to reduce such reduction in blood pressure and/or prevent or improve reduced renal function resulting from such reduction in blood pressure.

[0167] In another aspect, the (co)medication is a class III anti-arrhythmic, a selective β -blocker, a digitalis glycoside, a vitamin K antagonist, or a selective β -2-adrenoceptor agonist.

[0168] In yet another aspect, the (co) medication is potassium, a benzo-derivative, a plain sulfonamide, magnesium and/or digitalis glycosides. In some aspects, the (co)medication is a

class III anti-arrhythmic, a selective β -blocker, a digitalis glycoside, a vitamin K antagonist, or a selective β -2-adrenoceptor agonist.

[0169] In other aspects, the (co)medication is potassium, a benzo-derivative, a plain sulfonamide, magnesium and/or digitalis glycosides. As the before mentioned drugs are potentially kidney damaging in subjects exposed to them, reducing the amount of these drugs results in a reduced risk of reduced renal function in these subjects.

[0170] While the current application may describe features as part of the same embodiment or as parts of separate embodiments, the scope of the present invention also includes embodiments comprising any combination of all or some of the features described herein and falling under the scope of the claims.

Examples

Example 1

Human recombinant Alkaline Phosphatase for sepsis-associated acute kidney injury

[0171] Sepsis-associated acute kidney injury (AKI) adversely affects long-term renal outcomes and survival. Administration of the detoxifying enzyme alkaline phosphatase improved renal function and survival in preclinical studies. We investigated the efficacy and safety of a human recombinant alkaline phosphatase (RecAP) (**FIG. 2**) in patients with sepsis-associated AKI.

[0172] In Part 1 of the adaptive phase 2a/2b STOP-AKI trial, patients were randomized to receive RecAP 0.4, 0.8, or 1.6 mg/kg, or placebo, once daily for 3 days to establish the optimal dose.

[0173] In Part 2, this dose was compared with placebo. The primary endpoint was time-corrected area under the curve of the endogenous creatinine clearance for days 1-7 (AUC_{1-7} ECC), with renal replacement therapy (RRT) as a key secondary endpoint. Longer-term renal function, major adverse kidney events (MAKE) on days 28, 60, and 90, and survival were also analyzed. Overall, 301 patients were enrolled. Compared with placebo (n=116), RecAP (1.6 mg/kg, n=111) did not significantly affect AUC_{1-7} ECC or RRT rates. However, ECC improvement on days 21 ($P=0.02$) and 28 ($P=0.01$) was more pronounced and composite MAKE at days 60 ($P=0.045$) and 90 ($P=0.03$) had a lower incidence in patients receiving RecAP. All-cause 28-day mortality was 14.4% in RecAP-treated patients versus 26.7% in placebo-treated patients ($P=0.02$). No differences in (serious) adverse events were observed between groups.

[0174] RecAP treatment resulted in enhanced and sustained recovery of renal function and better survival versus placebo. RecAP appeared safe and well tolerated.

METHODS

I. STOP-AKI trial design and participants

[0175] The STOP-AKI trial, was an international randomized, double-blind, placebo-controlled, four arm, parallel-group, dose-finding adaptive phase 2a/2b trial conducted in critically ill adults with SA-AKI. The protocol, including slight changes in eligibility criteria implemented after inclusion of 120 patients, was published previously (Peters et al. BMJ Open 2016;6:e012371).

[0176] ICU patients aged ≥ 18 years with a diagnosis of sepsis (Levy et al. Crit Care Med 2003;31:1250-6) and a first diagnosis of AKI (Mehta et al. Crit Care 2007;11:R31) were eligible for study participation. The inclusion and exclusion criteria are provided below

Inclusion criteria

[0177] To be included the study, the subject

1. (i) has informed consent form signed by patient or legal representatives or independent investigator, according to local rules and regulations,
2. (ii) is aged 18-85 years, inclusive,
3. (iii) is admitted to the intensive care unit or intermediate care unit,
4. (iv) has diagnosis of sepsis (<96 hours prior to first study drug administration or <72 -hour prior to acute kidney injury (AKI) diagnosis), according to criteria defined by the American College of Chest Physicians/Society of Critical Care Medicine (Bone et al. Chest 1992;101:1644-55.), based on
 1. A. a proven or strongly suspected bacterial infection
 2. B. at least two of the following four SIRS criteria within a timeframe of 72 hours at the time of AKI diagnosis (Note: it is not required that symptoms are present simultaneously at study randomisation)
 1. 1. Core temperature $>38^{\circ}\text{C}$ or $<36^{\circ}\text{C}$
 2. 2. Heart rate >90 bpm (unless the patient has a medical condition known to increase heart rate or is receiving treatment to prevent tachycardia)
 3. 3. Respiratory rate >20 breaths/min, $\text{PaCO}_2 <32$ mm Hg or the use of mechanical ventilation for an acute respiratory process
 4. 4. White cell count $>12,000/\text{mm}^3$ or $<4000/\text{mm}^3$ or a differential count showing $>10\%$ immature neutrophils
5. (v) has first diagnosis of AKI, defined as AKI Stage 1 or greater, according to the

following AKIN criteria (Note: adjusted in regard to time-window):

1. A. Urinary output <0.5 ml/kg/h for >6 hours following adequate fluid resuscitation when applicable, in the absence of underlying primary renal disease, or
2. B. Increase (absolute) in serum creatinine >26.2 $\mu\text{mol/l}$ (0.30 mg/dl) compared with a serum creatinine value within the previous 48 hours, or presumed to have occurred in the previous 48 hours when compared with a reference creatinine value (see below), or
3. C. Increase (relative) in serum creatinine to $>150\%$ (>1.5 -fold) compared with a serum creatinine value in the previous 48 hours or presumed to have occurred in the previous 48 hours, when compared with a reference creatinine value (in the absence of primary underlying renal disease).

The reference creatinine value is a serum creatinine value in the following order of preference:

1. 1. Lowest value within 3 months of the hospital admission. If not available:
 2. 2. At hospital admission. If not available:
 3. 3. At ICU admission. If not available:
 4. 4. Lowest value between 3 and 12 months prior to hospital admission
6. (vi) when the diagnosis of AKI is made according to one of the AKIN serum creatinine criteria, continuing AKI needs to be confirmed by a confirmative fluid-corrected serum creatinine measure, defined as no decrease in serum creatinine ≥ 26.2 $\mu\text{mol/l}$ (≥ 0.30 mg/dl). The result must be available prior to randomisation. Administration of study treatment must be started within 24 hours after first AKI diagnosis
7. (vii) when the AKI diagnosis was made according to the AKIN urine output criteria, the oliguria or anuria should still meet the AKIN urine output criteria prior to randomisation. Administration of study treatment must be started within 24 hours after first AKI diagnosis

Exclusion criteria

[0178] Subjects were excluded from the study if at least one of the following conditions was fulfilled:

1. (i) Woman of childbearing potential with a positive pregnancy test (blood or urine), pregnant or breast feeding
2. (ii) Weighs more than 115 kg (253 lb)
3. (iii) Has life-support limitations (eg, do not intubate, do not dialyse, do not resuscitate)
4. (iv) Is known to be HIV-positive
5. (v) Has urosepsis. As urogenital obstruction, frequently observed during urosepsis, may also account for an increase in serum creatinine levels, it is not possible to define which proportion might be due to SA-AKI. Therefore, urosepsis patients are excluded.
6. (vi) Is already on renal replacement therapy (RRT) or a decision has been made to

initiate RRT within 24 hours after planned start of study drug administration

7. (vii) Is receiving immunosuppressant treatment or is on chronic high doses (high-dose therapy exceeding 2 weeks of treatment) of steroids equivalent to prednisone/prednisolone 0.5 mg/kg/day, including solid organ transplant patients. Patients with septic shock treated with hydrocortisone (eg, 3×100 mg) can be included
8. (viii) Is expected to have rapidly fatal outcome (within 24 hours)
9. (ix) Has known, confirmed fungal sepsis
10. (x) Has advanced chronic liver disease, confirmed by a Child-Pugh score of 10-15 (Class C)
11. (xi) Has acute pancreatitis with no established source of infection
12. (xii) Has participated in another investigational study within 30 days prior to enrolment into the study
13. (xiii) Is not expected to survive for 28 days due to medical conditions other than SA-AKI, including cancer (previous haematological malignancies that are not actively treated allowable), end-stage cardiac disease, cardiac arrest requiring cardiopulmonary resuscitation or with pulseless electrical activity or asystole within the past 30 days, end-stage lung disease and end-stage liver disease
14. (xiv) Has known prior history of CKD with a documented eGFR <60 ml/min by a commonly used formula such as MDRD or CKD-EPI, known GFR <60 ml/min or a known history of persistent creatinine level $\geq 150 \mu\text{mol/l}$ (1.70 mg/dl) prior to entry for reasons other than the current sepsis condition. CKD is a disease with a distinct pathophysiology compared with AKI. Also, if eGFR is impaired, the chances of an intervention to prevent further deterioration are limited. To increase homogeneity of the study population, these patients are therefore excluded.
AKI, acute kidney injury; AKIN, acute kidney injury network; CDK, chronic kidney disease; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; eGFR, estimated glomerular filtration rate; ICU, intensive care unit; IV, intravenous; MDRD, Modification of Diet in Renal Disease; NSAIDs, non-steroidal anti-inflammatory drugs; RRT, renal replacement therapy; SIRS, systemic inflammatory response syndrome.
15. (xv) Has diagnosis of malaria or other parasite infections
16. (xvi) Has burns on >20% of body surface
17. (xvii) Has had AKI diagnosis according to the AKI inclusion criteria for a period longer than 24 hours prior to study drug administration
18. (xviii) Is anticipated to be treated with non-continuous RRT from day 1 to day 7
19. (xix) During day 1 to day 7, continuous RRT is anticipated to be started or stopped not according to per protocol criteria
20. (xx) The AKI is most likely attributable to other causes than sepsis, such as nephrotoxic drugs (NSAIDs, contrast, aminoglycosides) and renal perfusion-related (acute abdominal aortic aneurysm, dissection, renal artery stenosis)
21. (xxi) Improvement in serum creatinine of at least $26.2 \mu\text{mol/l}$ (0.30 mg/dl) prior to administration of the study drug
22. (xxii) Patients who use nephrotoxic medication and who fulfil the SA-AKI inclusion criteria at screening are not eligible if the use of this nephrotoxic medication is planned to continue (eg, NSAIDs, ACE inhibitors, gentamycin, tobramycin) when alternative,

medically appropriate non-nephrotoxic medication is available.

- 23. (xxiii) Has a history of known intravenous drug abuse
- 24. (xxiv) Is an employee or family member of the investigator or study site personnel
- 25. (xxv) Has active hematological malignancy

[0179] Treatment was initiated within 24 hours after SA-AKI was first diagnosed and within 96 hours following fulfilment of sepsis criteria (Levy et al. Crit Care Med 2003;31:1250-6.). Patients enrolled during the first part of the study were randomly assigned to receive either placebo or one of three RecAP doses (0.4; 0.8; 1.6 mg/kg) once daily for 3 days using an 1:1:1:1 allocation ratio.

[0180] After the 120th patient, futility or superiority of the intervention on the primary endpoint data and the fit to an assumed maximum effect attributable to the drug ($E_{\max}$) model dose-response curve was assessed. An interim analysis proceeded as follows.

[0181] *Interim analysis:* An unblinded interim analysis was conducted on the Part 1 data to determine the optimal RecAP dose for Part 2. This analysis compared the primary efficacy endpoint and a selection of the safety data for the four treatment groups from Part 1. The interim analysis was conducted when the first 7 days of laboratory data had been collected for 120 patients from Part 1, unless the patient was randomized but had died or discontinued before completing 7 days.

[0182] To maintain the blind, the interim analysis was conducted and delivered by an unblinded PPD biostatistics team located at a different site to the blinded PPD biostatistics personnel involved in the study. A futility analysis was conducted at the interim analysis.

[0183] Futility analysis: At the interim analysis, the data were reviewed to identify whether none of the RecAP doses show evidence of efficacy. The results were assessed from the comparison of each RecAP dose with placebo on the primary endpoint, using a one-sided significance level of 0.8. This significance level was chosen for the futility analysis to ensure a sufficient overall power for the study and to allow for the multiple comparison to placebo. At the futility analysis, each RecAP dose would only be deemed as having shown some evidence of efficacy if the one-sided, unadjusted p-value for its comparison with placebo is less than 0.8.

[0184] *Selection of optimal RecAP dose:* The optimal dose was defined as the dose with the best safe improvement on creatinine clearance compared to placebo.

[0185] Unless the DSMB had safety concerns pertaining to specify dose level(s) of RecAP and/or the $E_{\max}$ model (dose-response modelling of the primary endpoint) highlights unusual trend(s), the optimal dose would be, preferably, the highest dose of RecAP (i.e. 1.6 mg/kg).

[0186] If there were safety concerns and/or some concerns with regard to the dose-response

curve, the selection of the optimal dose would be carried out using the following effectiveness criteria. The optimal dose level would be selected amongst the dose levels where there are no safety or/and dose-response modelling concerns.

[0187] The effectiveness of a dose level would be determined based on the one-sided unadjusted p-value for comparison with placebo on the primary endpoint, i.e. the dose with the smallest p-value would be considered the most effective dose. Any dose with a one-sided unadjusted p-value less than 0.025 would be considered to have a significantly greater improvement on creatinine clearance than placebo. However, the comparison with placebo did not have to be significant at the 2.5% level for a dose to be considered the most effective dose. Should more than one dose have appeared to be the most effective with the same effect, the safety data as well as the Emax dose-response modelling should be closely examined for selecting the optimal dose.

[0188] The adaptive design directed the cessation of two treatment arms, as described in the statistical analysis plan such that, in Part 2, patients were randomly assigned to receive either placebo or the optimal dose of RecAP selected based on interim analysis of the primary endpoint data.

II. Randomization and study medication

[0189] The randomization schedule was stratified by site. Study drug dose rationale (Peters et al. BMJ Open 2016;6:e012371) is explained below.

Dose Rationale and Pharmacokinetics

[0190] Selection of Doses in the Study and Timing of Doses for Each Patient: No dose-limiting toxicity was observed in pre-clinical and clinical studies with RecAP up to daily doses of 18 mg/kg (10 000 U/kg) and 36 mg/kg (20 000 U/kg) maintained for 14 days in Göttingen mini-pigs, and in healthy volunteers for a single dose up to 3.2 mg/kg (2000 U/kg) and for multiple doses up to 3 daily doses of 1.6 mg/kg (1000 U/kg). Therefore, the dose rationale was primarily based on: 1) observed Phase 1 RecAP PK parameters; 2) trough levels (293 U/l, corresponding to 0.171 mg/l RecAP) obtained in a previous study using Bovine Intestinal Alkaline Phosphatase (BiAP); and 3) exposure maintained during the previous study using BiAP and associated with clinical activity. The three dose-level schedules for the first part of this Phase 2 study, in addition to the placebo group, are shown in **FIG. 1**.

[0191] Using PK simulation experiments, these schedules provided the area under the concentration-time curve from zero up to an infinite time ($AUC_{0-\infty}$) and observed minimum post-administration plasma or serum concentration (C_{min}) levels that were similar to or exceeded the presumed minimal requirements based on two different BiAP studies.

Furthermore, these dose groups were likely to achieve significantly different exposures when comparing three groups of 30 patients (as informed by the observed PK variability in Phase 1 patients). Analyzing PK simulations, the RecAP low-dose group was expected to achieve sufficient exposure, with C_{min} dropping below target trough concentration before re-infusion, whereas the mid-dose and high-dose groups exceeded target exposure and C_{min} . If clinical activity was observed to be different in the Phase 2 study of STOP-AKI at interim analysis, the proposed design of dose groups was likely to enable a clear decision on optimal RecAP dose for continuation into the second part of the study.

[0192] Pharmacokinetics: The PK model developed in phase I is still applicable, indicating similar pharmacokinetic properties. The PK model allows for a co-variate analysis. Co-variables studied include: age, weight, BMI, race, AKIN stage, RRT, baseline CrCl, KIM, CRP, IL6, IL18, LBP, FeNa, BUN, albumin, ALAT, ASAT, and bilirubin. Except for ALAT and ASAT at baseline, none of these parameters appears to have an influence on the PK of RecAP. See **FIG. 6**.

[0193] The lowest dose was selected in order to reach trough plasma concentrations below the estimated effective concentration. The middle and highest doses were selected to have trough concentrations at or in excess of the estimated effective concentration, respectively. Study drug was administered as a 1-hour intravenous infusion within 24 hours after SA-AKI was first diagnosed, and then 24 ± 1 and 48 ± 1 hours later. Administration of nephrotoxic drugs were avoided where possible, as recommended (Okusa et al. *Kidney Int* 2014;85:39-48). Personnel involved in this study were blinded to treatment assignment during Parts 1 and 2; therefore, to maintain blinding, AP levels were not measured until day 14 (details on blinding are shown below).

[0194] Blinding: RecAP was supplied as a clear, colorless, pyrogen-free solution for intravenous infusion with a content of 40 mg per vial at 8.0 mg/ml in an aqueous buffer. Matching placebo was supplied in an identical manner. All ampoules and drug boxes had a fully identical appearance and label. All persons involved in the study were blinded to treatment assignment. The randomization schedule was held by an independent team at a different regional location and was not revealed to the blinded study team until all patients had completed the study and the database had been finalized for the end of the study. The determination of AP activity, often part of the routine clinical chemistry panel, could lead to unblinding and to erroneous interpretation of liver function, as the RecAP administered would increase the AP activity, exceeding many times the reference range AP levels. For that reason, AP activity levels from samples taken during the first 14 days of the study were not allowed to be reported to the study team members or to any other study staff member involved in the conduct of the study. After day 14, considering the terminal half-life of RecAP, AP activity levels were expected to have returned to normal levels. Therefore, results of AP activity in samples that were taken after day 14 were reported.

IV. Outcome measures

[0195] The primary objective of this study was to investigate the optimal therapeutic dose of RecAP and to evaluate its effect on renal function (ECC), defined as the area under the time-corrected (i.e. measured per day) curve (AUC) for ECC from day 1 through 7, divided by 7 to provide a mean creatinine clearance (AUC_{1-7} ECC). Requirement for renal replacement therapy (RRT) was the main related clinical parameter. Sites were advised to follow criteria for the initiation and termination of RRT (Bellomo et al. The Lancet 2012;380:756-66.). Other renal endpoints included blood urea nitrogen (BUN) clearance, RRT-free days, renal function (ECC and estimated glomerular filtration rate (eGFR)) and MAKE composite scores at days 28 (RRT or death<28 days), 60 (eGFR<60 ml/min, or chronic RRT, or death<60 days), and 90 (eGFR<60 ml/min, or chronic RRT, or hospitalization for new episode of AKI, or death<90 days). See **FIG. 3**.

[0196] Safety, tolerability, pharmacokinetics (in the first 120 patients), immunogenicity, systemic and urinary biomarkers, and the effect of RecAP on quality of life in patients with SA-AKI were also investigated. A-priori subgroup analyses were planned to determine whether RecAP treatment demonstrates different levels of efficacy in specific patient groups. All endpoints, including non-safety and non-renal secondary end points are described in detail below.

Study Endpoints

[0197] Primary Endpoint: The primary endpoint was the area under the time-corrected endogenous creatinine- clearance (ECC) curve from Day 1 to Day 7 (AUC_{1-7}), calculated as the average of the standardized endogenous creatinine clearance values over the 7 days. Urine was collected for 5-7 hours and the ECC was calculated from the urine and serum creatinine concentration and urine volume. The ECC was determined daily from day 1 to day 7. The complete AUC was calculated and divided by 7 to produce a mean ECC.

Secondary Endpoints

[0198]

1. (a) Key Secondary Endpoint: The key secondary endpoint was RRT incidence during the period from day 1 (after first treatment) to day 28, inclusive.
2. (b) Other Secondary Endpoints:

Renal

[0199] Renal endpoints included the following:

- Volume of urine (daily from day 1 to day 7, inclusive, and on subsequent visit days if reliable 6-hour urine collection was possible) normalized per hour.
- Serum creatinine and BUN/urea (daily from day 1 to day 7, inclusive, and on subsequent visit days).
- BUN/LTrea clearance (daily from day 1 to day 7, inclusive, and on subsequent visit days if reliable urine collection was possible).
- Peak value of serum creatinine and peak values of BUN/urea (during the period from day 1 to day 7, inclusive).
- RRT-free days: Days alive and not dependent of RRT from randomization to day 28, inclusive. Support-free days were calculated as described here. In the Statistical Analysis Plan, support-free days were defined as 'a day on which a patient did not receive support' (RRT/mechanical ventilation/vasopressor/inotropic) in which the last observation was carried forward in case of death or withdrawal.
- Total number of days on RRT (during the period from day 1 to day 28, inclusive). A day on RRT was defined as a day on which a patient received any form of RRT (including RRT with interruptions) for any period of time on that day.
- Reasons for initiation of RRT (during the period from day 1 to day 28, inclusive).
- Kidney function at days 14, 21, and 28 as assessed by measured creatinine clearance if available, otherwise as assessed by eGFR (estimated by the CKD-EPI formula based on serum creatinine concentration).
- Kidney function at day 60 and day 90 as assessed by eGFR (estimated by the CKD-EPI formula based on serum creatinine concentration).
- Sustained loss of kidney function at day 60 and day 90, defined by eGFR <60 mL/min (with eGFR estimated by the CKD-EPI formula based on serum creatinine concentration).
- Incidence of dialysis dependency at day 60 and day 90.

Other Organs

[0200] Endpoints for organs other than kidney included the following:

- Liver enzymes (aspartate aminotransferase [AST], alanine aminotransferase [ALT], gamma-glutamyl transpeptidase [GGT], lactate dehydrogenase [LDH], bilirubin) with the exception of AP.
- Lung function as assessed by fraction partial pressure arterial oxygen (PaO₂)/fraction of inspired oxygen (FiO₂) (P/F ratio) Carrico index, positive end expiratory pressure (PEEP), and tidal volume in mechanically ventilated patients.
- Mechanical ventilator-free days: Days alive and not dependent of mechanical ventilation from randomization to day 28, inclusive#. A ventilator-free day was defined as a day on

which a patient was not on ventilator (invasive or non-invasive mechanical ventilation).

- Time from start of first administration of study drug to being off mechanical ventilator (from day 1 to day 28, inclusive) for those patients who were on mechanical ventilator at the start of this period.
- Shock-free days. Days alive and not dependent of vasopressor/inotropic therapy from randomization to day 28, inclusive. A patient was considered to be shock free if he or she was not on vasopressors or inotropic agents (including, but not limited to noradrenaline, adrenaline, dobutamine, dopamine, vasopressin, or enoximone). Support-free days were calculated as described here. In the Statistical Analysis Plan, support-free days were defined as 'a day on which a patient did not receive support' (RRT/mechanical ventilation/vasopressor/inotropic) in which the last observation was carried forward in case of death or withdrawal.
- Time from start of first administration of treatment to being shock free (from day 1 to day 28, inclusive) for those patients who were not shock free at the start of this period.
- Sequential organ failure assessment (SOFA) scores during ICU/intermediate care stay.
- Number of dysfunctional organs as assessed by SOFA scores (from Baseline to day 28, inclusive).
- Deaths during the 90-day study period (by recording date).

Biomarkers

[0201] Biomarker endpoints included the following:

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

Other Endpoints

[0202] Additional endpoints included the following:

- Three major adverse kidney events (MAKE) composite endpoints: patients who met, or did not meet, at least one of the following criteria:

- MAKE composite endpoint 1: Received RRT or died (before day 28 [inclusive]).
- MAKE composite endpoint 2: eGFR <60 mL/min at day 60 (estimated by the CKD-EPI formula); or dialysis dependency; or died (before day 60).
- MAKE composite endpoint 3: eGFR <60 mL/min at day 90 (estimated by the CKD-EPI formula); or dialysis dependency; hospitalized for a new episode of AKI (at day 90), or died (before day 90).
- Serology as assessed by immunoglobulin G (IgG), immunoglobulin E (IgE), and total immunoglobulin.
- Safety parameters, including (serious) adverse events, laboratory assessments (clinical chemistry, hematology, and urinalysis parameters not considered in the efficacy analysis), vital signs, and ECG data.
- Quality of life, assessed by EQ-5D questionnaire at baseline, ICU/intermediate care discharge, and day 90.
- Time from start of first administration of treatment to discharge from ICU/intermediate care, where discharge was defined as the time when the decision was made to transfer the patient (as opposed to the time of actual transfer, that could be delayed due to placement issues).
- Total time in ICU/intermediate care from the start of first administration of study drug (during the period Day 1 to Day 28 inclusive and during the period from day 1 to day 90, inclusive) using the time of actual transfer.
- Time from start of first administration of treatment to discharge from hospital, where discharge was defined as the time when the decision was made to transfer the patient (as opposed to the time of actual transfer).
- Total time in hospital from the start of first administration of study drug (during the period from day 1 to day 28, inclusive, and during the period day 1 to day 90, inclusive) using the time the decision was made to discharge the patient.

V. Statistical analysis

[0203] Data were analyzed according to the intention-to-treat principle for patients from whom informed consent was obtained and who were randomized to a treatment arm. Details related to the sample size calculations are described below.

Sample Size Calculations

[0204] A sample size was planned of $n_1=30$ patients per treatment group in Part 1 with an additional $n_2=85$ patients recruited to the optimal RecAP-dose and placebo treatment groups in

Part 2 (for a total sample size of $n=290$ patients). Custom-programmed simulations were performed using SAS software V.9.2 to determine power and type I error rate of the chosen sample size and design in a number of different dose-response scenarios. Each scenario assumed a standard deviation of 49 ml/min for the primary end point with an assumed response of 60 ml/min for the placebo group, and between 60 mL/min (no treatment effect) and 79 ml/min (strong treatment effect) for the RecAP dose groups.

[0205] Fifty thousand simulations were performed to show that the one-sided type I error rate is 2.4% (and hence is well controlled at the one-sided 2.5% significance level). The power was defined as the probability of rejecting the null hypothesis (of no difference between treatment groups) when one or more RecAP dose groups have a treatment effect, defined as a response of 69.5 ml/min. This was investigated across seven scenarios with 10 000 simulations performed for each. The chosen design achieved power of between 79% and 86% for scenarios with strong treatment effects for the medium and high RecAP dose groups; a varying response for the low-dose group, and between 66% and 67% when only the high-dose group had a strong treatment effect. As the sample size determination was based on the number of patients required for the intention-to-treat (ITT) analysis, patients who are randomly assigned and subsequently withdrew prior to completion of the study were not to be replaced.

[0206] A per-protocol analysis compared the intervention groups with the placebo group for patients who received study medication according to the study protocol, and had no more than two missing ECC values on days 1 through 7, as detailed in the statistical analysis plan.

[0207] For the descriptive statistics, continuous variables are presented as mean with standard deviation or median with interquartile range, depending on their distribution. Normally distributed variables were compared using Student's t test; Mann-Whitney U tests were used to compare non-normally distributed variables. Categorical (and binary) variables are presented as numbers with percentages and analyzed using chi-squared tests. Survival analyses with Kaplan-Meier curves were used for graphical presentation. Cox proportional-hazard regression analyses were used to estimate the hazard ratio for survival and for the number of RRT-, shock-, and mechanical ventilation-free days during study days 1-28 with the use of RecAP versus placebo.

[0208] The analysis of the primary efficacy endpoint was performed by an analysis of variance. All analyses performed on the secondary endpoints were for exploratory purposes only, therefore no multiplicity adjustment was required. A post-hoc multivariate analysis was conducted to establish the robustness of the RecAP effect. All statistical tests (SAS software version 9.4; SAS Institute Inc., Cary, NC, U.S.A.), performed on the intention-to-treat population, were two-sided using a 5% significance level.

RESULTS

I. Participants

[0209] Of 326 patients who passed an initial screen, 301 patients were enrolled (**FIG. 4**) across 53 sites in 11 countries in the European Union and North America. Patients received RecAP 0.4 mg/kg, (n=30), 0.8 mg/kg, (n=32), 1.6 mg/kg (n=29 in Part 1 and n=82 in Part 2) or placebo (n=30 in Part 1 and n=86 in Part 2). Randomization resulted in well-balanced demographic and patient characteristics, except for slight baseline differences in renal function between groups (**FIG. 5**).

II. Efficacy outcomes

[0210] In the dose-finding Part 1 of the trial, the median AUC₁₋₇ ECC was 47.0, 63.5, 60.7 ml/min in patients treated with RecAP 0.4, 0.8, 1.6 mg/kg, respectively, compared with 46.2 ml/min in the placebo group. See **FIG. 7**.

[0211] Following the interim analysis, the DSMB advised continuing the study with RecAP 1.6 mg/kg. As per the statistical analysis plan, only the effects of the RecAP 1.6 mg/kg group were compared with those of the placebo group in the primary analyses. When data from the RecAP 1.6 mg/kg groups from Parts 1 and 2 were combined (n=111), no significant difference was observed in the primary efficacy outcome (AUC₁₋₇ECC) for RecAP treatment compared with placebo (n=116). Furthermore, the requirement of RRT was not significantly affected by RecAP treatment (**FIG. 8**).

[0212] As study drug infusion started as soon as possible following the AKI diagnosis, in less than half of the patients clearance data was obtained on day 0. In **FIG. 9A** and **FIG. 9B**, the difference in ECC and BUN clearance was calculated from day 1 onwards. Improvement of ECC (P=0.036) (**FIG. 9A**), as well as BUN clearance (P=0.033) (**FIG. 9B**), over 28 days was enhanced in RecAP-treated patients compared with those receiving placebo, based on differences that occurred from day 21 onwards.

[0213] Treatment with RecAP did not affect MAKE₂₈, but resulted in a significantly lower incidence of MAKE at day 60 (P=0.045) and day 90 (P=0.031) compared with placebo, (**FIG. 8**).

[0214] All-cause mortality by day 28 was statistically significantly lower in the RecAP group versus the placebo group (14.4% vs 26.7%, respectively; P=0.022). **FIG. 10** shows mortality through to day 90 (favored treatment, P=0.030). A similar survival benefit was observed in the RecAP 0.8 mg/kg dose group. See **FIG. 11**, which presents a Kaplan Meier survival curve for all groups.

[0215] Inflammatory and renal injury biomarkers, as well as other non-renal endpoints were not significantly influenced by RecAP treatment. See **FIG. 12** (table presenting primary and

secondary endpoints for all groups) and **FIGS. 13A to 31E**.

[0216] Per protocol analyses yielded comparable results.

[0217] To test the robustness of the RecAP-mediated effect on survival, a post-hoc multivariate analysis was conducted, including Sequential Organ Failure Assessment (SOFA) subgroups and study arm as covariates. Treatment with RecAP remained significantly related to survival (hazard ratio 0.50, 95% CI 0.28-0.90; $P=0.02$; see **FIG. 14**).

III. Subgroup analyses

[0218] No statistically significant effects on the primary endpoint and key secondary endpoint were observed in the a-priori defined subgroups. See **FIGS. 15A to 15L**. Baseline and day-1 ECC strongly correlated with AUC_{1-7} ECC [$r=0.88$, common slope 1.023 (95% confidence interval [CI] 0.946-1.100); $P<0.0001$], indicating the relevance of initial renal function.

IV. Safety

[0219] Treatment emergent Serious Adverse Events were reported in 43% of patients that received RecAP 1.6 mg/kg and in 50% of patients that received placebo. See **FIG. 16**. No RecAP dose-dependency in the incidence and nature of Adverse Events (AE's) was observed. Anti-drug antibody titers were just above the detection limit in 9 patients treated with RecAP.

DISCUSSION

[0220] In this multinational double-blind, randomized controlled trial involving patients with SA-AKI, we observed that addition of RecAP to standard of care had a large impact on survival, associated with an improved and sustained recovery of renal function and longer-term clinical outcomes, including composite MAKE endpoints. Because this was a dose-finding proof-of-principle phase 2 trial, it was designed to include endpoints related to both renal dysfunction (e.g. short-term ECC) and the long-term, more patient-centered, clinical outcomes (e.g. $MAKE_{day60-90}$, survival).

[0221] Function is a competing endpoint with death and may therefore differ from clinical outcome (Billings et al. Nephron Clinical practice 2014;127:89-93). In the current study, no penalty for death on functional measures was used, so separation of survival curves makes it harder to account for function in non-survivors, which may explain the discrepancy between the short- and longer-term therapeutic efficacy observed.

[0222] In sepsis, injury is known to be heterogeneous throughout the kidney with patchy

tubular damage being the most common histological finding (Kellum et al. *Nat Rev Nephrol* 2018). By contrast, renal function is a global measure. Consequently, acute measures of organ function may belie underlying organ damage that only becomes evident later. RecAP reduced kidney damage and facilitated organ function could manifest after weeks. Several additional reasons are plausible for the observed RecAP on short-term ECC and its positive effects on the longer-term clinical outcomes. First, creatinine and its clearance are recognized to be of limited precision to estimate renal function (Shemesh et al. *Kidney International* 1985;28:830-8; Perrone et al. *Clin Chem* 1992;38:1933-53), however no feasible alternative is currently available. Second, despite randomization, there was a slight imbalance in renal function between groups. Previous work (Kellum et al. *Am J Respir Crit Care Med* 2017;195:784-91), as well as the current trial, illustrates that the degree of initial renal dysfunction dictates the extent of renal recovery and survival, as more severe kidney failure correlates with worse outcomes. Therefore, the somewhat more impaired renal function may account for the absence of an improvement of ECC in the first week. Third, statistically significant differences in recovery of ECC between treatment groups emerged from day 21 onwards. Fourth, the significantly higher survival rate in the RecAP-treated group likely affected the primary and key secondary outcomes, as poor renal function in the most severely ill, but surviving, patients in the treatment group may have attenuated the ECC. Of interest, the survival benefit in the treatment arm did not negatively affect any of the other endpoints, such as quality of life. To further explore the robustness of the effect of RecAP on survival we performed a post-hoc multivariate analysis including SOFA-subgroups, known to influence survival. In this analysis, randomization to the treatment arm remained a strong predictor of survival.

[0223] In the current larger trial, we evaluated a human recombinant AP that is suitable for widespread clinical use. It remains unclear to what extent renal protection is mediated through direct effects on tubular cells or systemic effects indirectly prevent damage to the kidneys, or whether kidney function improves as general effect of improving sepsis. On one hand, AP is depleted in the kidney following an ischemic insult (Khundmiri et al. *Am J Physiol* 1997;273:849-56), and RecAP attenuates the inflammatory response in isolated human proximal tubule cells (Peters et al. *Br J Pharmacol* 2015;172:4932-45). On the other hand, systemic detoxifying properties may lead to more swift normalization of circulating inflammatory markers (Pickkers et al. *Crit Care* 2012;16:R14) indirectly benefiting the kidneys. It appears that direct effects on renal tissue are important. Treatment with RecAP can attenuate renal inflammation and thereby influences its detrimental long-term effects, and/or RecAP may possibly enhance renal recovery from AKI opposed to prevention of the primary injury itself.

[0224] In conclusion, RecAP therapy was considered safe and well-tolerated. In sepsis patients with AKI, RecAP treatment was observed to provide significant improvements of longer-term renal function, MAKE₆₀₋₉₀, and survival.

[0225] A post-hoc analysis in which patients were stratified according to the severity of kidney disease defined according to baseline ECC or baseline eGFR revealed distinct effects resulting from the administration of RecAP to patients with moderate to severe injury and for patients with severe kidney injury. See **FIG. 17**. Mild SA-AKI did not increase mortality in patients, and

administration of AP appears to have had no effect on the survival of that population. The same was observed on the moderate to severe SA-AKI group. Surprisingly, patients that presented with a baseline eGFR <60 ml/min benefit from AP administration with regard to mortality similarly as the ECC <15 ml/min group. See FIG. 22. On the other hand, individuals with severe SA-AKI (as determined by ECC <15 ml/min) treated with AP had a survival rate comparable to both AP treated or placebo treated moderate to severe injury and mild injury groups. In contrast, SA-AKI patients treated with placebo has a much lower survival rate. The treatment of patients with severe SA-AKI with 1000 U/kg doses of RecAP during three days after the onset of sepsis resulted in a dramatic survival increase (>20%) up to 90 days after treatment. Furthermore, statistically significant increases in kidney function (increased creatinine clearance) were observed in the moderate to severe SA-AKI at days 21 and 28 after treatment. An increase in kidney function was observed the severe SA-AKI group. See also **FIGS. 18, 19 and 20**. The model fit statistics presented in **FIG. 18** such as R squared and Log likelihood Ratio (LR) test demonstrate a comparable but slightly better fit for the continuous model (Model 1) compared to the categorical model (Model 2) with one less parameter; the categorical model however provides coefficients that are interpretable as hazard ratios (HR).

Example 2

Determination of RecAP Enzyme Activity and Protein Concentration

Activity assay:

[0226] Determination of recAP enzyme activity was based on the conversion (hydrolysis) of 4-nitrophenolphosphate into 4-nitrophenol which is colored yellow. The change in optical density at 405nm per unit time is a measure for the alkaline phosphatase activity. The assay buffer consisted of 0.25M Glycine buffer pH 9.6 at 25°C with 2mM MgCh and 0.1 mM ZnCh and 8.5mM 4-nitrophenolphosphate.

[0227] The unit (U) definition for recAP, expressed as U/mL, is the amount of enzyme causing the hydrolysis of 1 µmol of 4-nitrophenolphosphate per minute at pH 9.6 and 25°C.

Protein concentration:

[0228] Determination of total protein concentration in RecAP Drug Substance and Drug Product was performed by UV/Vis analysis. A RecAP solution is analysed at 280nm and the absorbance is a measure for protein content (mg/mL) using the formula:

Concentration (mg/mL) = $[A / (a \times b)] \times DF$ with $A=A_{280}$; b = path length; a = mass extinction

coefficient of 1.01 mL mg⁻¹ cm⁻¹; DF is dilution factor.

REFERENCES CITED IN THE DESCRIPTION

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This list of references cited by the applicant is for the reader's convenience only. It does not form part of the European patent document. Even though great care has been taken in compiling the references, errors or omissions cannot be excluded and the EPO disclaims all liability in this regard.

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PATENTKRAV

1. Alkalisk phosphatase (AP) til anvendelse i en fremgangsmåde til behandling af sepsis-forbundet akut nyresygdomslæsion (SA-AKI) hos et individ med behov derfor, og som omfatter administration af en virksom mængde af AP til individet, hvor
 - (i) individet har en eGFR (estimeret glomerulær filtreringshastighed) < 60 ml/min før behandling med AP,
 - (ii) AP administreres i mindst én dosis på 500 U/kg til 2.000 U/kg, og
 - (iii) administrationen af AP resulterer i en stigning i nyrefunktion.
2. AP til anvendelse ifølge krav 1, hvor individet har 15 - 60 ml/min eGFR før behandling med AP.
3. AP til anvendelse ifølge krav 1, hvor individet har <15 ml/min eGFR før behandling med AP.
4. AP til anvendelse ifølge et hvilket som helst af krav 1 til 3, hvor AP er en human AP.
5. AP til anvendelse ifølge et hvilket som helst af krav 1 til 4, hvor AP er en rekombinant AP.
6. AP til anvendelse ifølge krav 5, hvor den rekombinante AP er kimærisk.
7. AP til anvendelse ifølge krav 6, hvor den kimæriske AP har mindst 80 %, mindst 85 %, mindst 90 %, mindst 95 %, mindst 96 %, mindst 97 %, mindst 98 %, mindst 99 % eller 100 % sekvensidentitet med aminosyresekvensen af RecAP (SEQ ID NO: 1).
8. AP til anvendelse ifølge et hvilket som helst af krav 1 til 7, hvor stigningen i nyrefunktion omfatter en stigning i endogen kreatininclearance (ECC) i forhold til ECC i fravær af behandling, eller en stigning i eGFR i forhold til eGFR i fravær af behandling.
9. AP til anvendelse ifølge et hvilket som helst af krav 1 til 8, hvor sepsis påvises mindre end 96 timer før AP-administration og/eller mindre end 72 timer før påvisning af SA-AKI.
10. AP til anvendelse ifølge et hvilket som helst af krav 1 til 9, hvor behandling initieres

inden 24 timer, efter at sepsis er påvist, og/eller inden 24 timer, efter at SA-AKI er påvist.

11. AP til anvendelse ifølge et hvilket som helst af krav 1 til 10, hvor AP administreres én gang dagligt eller i tre daglige doser.

5

12. AP til anvendelse ifølge et hvilket som helst af krav 1 til 11, hvor AP administreres intravenøst.

13. AP til anvendelse ifølge et hvilket som helst af krav 1 til 12, hvor AP-dosen er 0,8 mg/kg eller 1,6 mg/kg RecAP og/eller 500 U/kg eller 1000 U/kg RecAP.

10

14. AP til anvendelse ifølge et hvilket som helst af krav 1 til 13, hvor administrationen af mindst én AP-dosis resulterer i en forkortelse af varigheden eller ophør af nyreerstatningsterapi (RRT) hos en person, der er i RRT.

15

15. AP til anvendelse ifølge et hvilket som helst af krav 1 til 14, hvor administrationen af mindst én AP-dosis resulterer i bevaring af eller stigning i glomerulær filtreringshastighed (GFR) eller eGFR hos individet.

DRAWINGS

Drawing

Dose Group	AUC _{0-inf} (h•U/L) Median [IQR]	Fold (over BiAP)	C _{min} (U/L) Mean (SD)	Fold (over BiAP)	T _{trough} (h)
250 U/kg or 0.40 mg/kg qd × 3	45762 [26225–75202]	2.29	C _{min1} : 120 (74 - 187) C _{min2} : 183 (113 - 287)	0.41	t _{trough1} : 7.2 t _{trough2} : 35.0 t _{trough3} : 63.2
500 U/kg or 0.80 mg/kg qd × 3	90901 [50782–156914]	4.55	C _{min1} : 241 (140 - 391) C _{min2} : 369 (213 - 599)	0.82	t _{trough1} : 18.7 t _{trough2} : NR t _{trough3} : 92.1
1000 U/kg or 1.60 mg/kg qd × 3	184347 [102688–301544]	9.22	C _{min1} : 486 (288 - 742) C _{min2} : 746 (443 - 1136)	1.66	t _{trough1} : NR t _{trough2} : NR t _{trough3} : 144
BiAP				2.54	
67.5 U/kg (10 min) + 132.5 U/kg/24 h for 48 h	20000 (estimated)	NA	293 ± 257 ('trough')	NA	NA

AUC_{0-inf} denotes area under the concentration-time curve from zero to an infinite time, BiAP bovine intestinal alkaline phosphatase, C_{min} observed minimum plasma or serum concentration after administration, NA not available; NR not recorded; qd every day (quaque die).
C_{min} was determined just before re-infusion (C_{min1} at t = 24 h; C_{min2} at t = 48 h). Trough was set at 293 U/L
T_{trough} reflected the time to reach trough (t_{trough1} after first infusion, t_{trough2} after second infusion, etc).

FIG. 1

SEQUENCE OF RECAP (SEQ ID NO: 1)

1 VTAAEDDPAFNNQAAEALDAKKELDTQVANNLTPLSDGLCVPTVTRILNQGHKELQETFLANDRFFVLADEKTVVDRCTQDAETATL

101 GNVKAPGCTGLSAAAPNOCSTTHNVEVGVNHPAQKSKGVVTTTNGHAFSACTVANTNNKWSAAEASAKQKQDSAGQLISNNDIVIL

201 GQKKVPT

301 RHHQGYLVYECRINDQNHQVAYQVTEAVDEDAIERAQGLISEETILLVTADSSHPTFOOYDLESGSEGLAPDQADKAYTLLYNGRQIV

401 LKQDARVYTESGSEYHQSAVPLDEETHQGLDIAVFAQIQALVHCFQDQFPAAVHAFNAQLEETFAQLALPACTTQ

FIG. 2

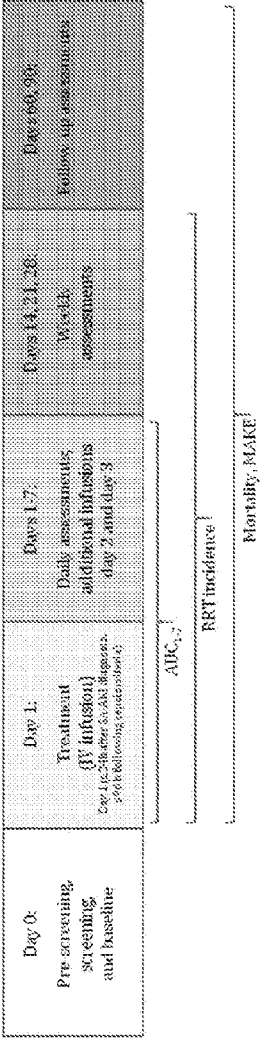


FIG. 3

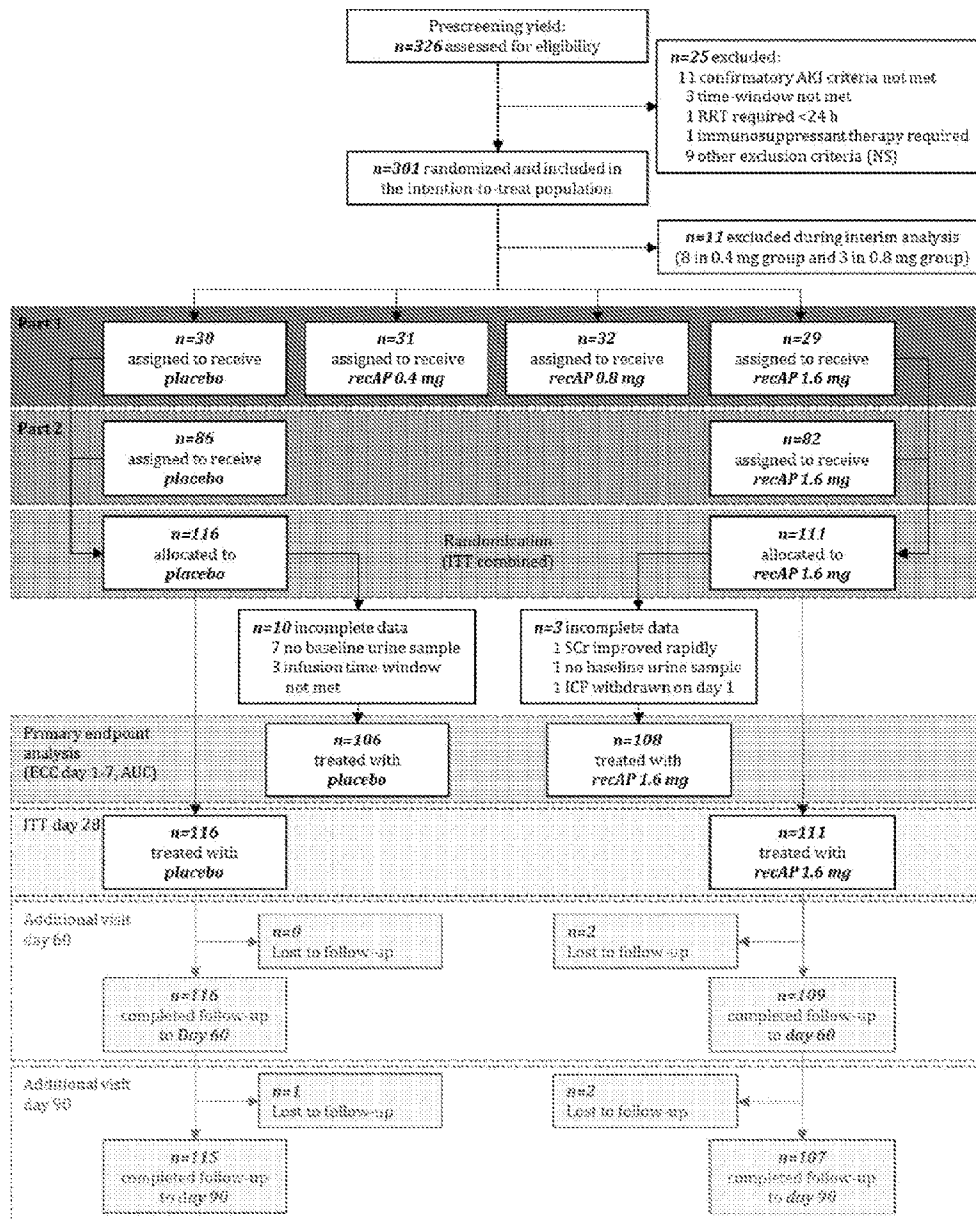


FIG. 4

Demographic and Baseline Disease Characteristics

Characteristic	recAP			
	Placebo (N=116)	0.4 mg/kg (N=31)	0.8 mg/kg (N=32)	1.6 mg/kg (N=111)
Total				(N=290)
Age, median [IQR] — years	68.0 [61.0 – 75.0]	67.0 [61.0 – 72.0]	66.5 [62.5 – 72.0]	67.0 [57.0 – 73.0]
Male — no. (%)	84 (72.4)	23 (74.2)	16 (50.0)	82 (73.9)
Caucasian — no. (%)	95 (81.9)	25 (80.6)	27 (84.4)	95 (85.6)
Weight, median [IQR] — kg	79.2 [70.0 – 86.5]	78.0 [70.0 – 86.0]	79.0 [72.0 – 90.0]	80.0 [71.8 – 89.1]
Height, median [IQR] — cm	174.0 [165.0 – 178.0]	172.5 [165.0 – 184.0]	167.0 [160.0 – 174.0]	173.0 [168.0 – 179.0]
BMI, median [IQR] — kg/m ²	26.3 [23.9 – 29.4]	25.8 [22.4 – 28.4]	27.4 [25.2 – 30.7]	26.6 [23.9 – 29.4]
Disease severity				
APACHE II score, median [IQR]	26.0 [20.0 – 33.5]	30.0 [25.0 – 35.0]	26.0 [20.0 – 34.5]	26.0 [21.0 – 33.0]
SAPS, median [IQR]	47.0 [39.0 – 60.0]	52.0 [42.0 – 70.0]	50.5 [38.0 – 60.5]	49.5 [40.0 – 63.0]
SOFA score, median [IQR]	10.0 [8.0 – 12.0]	10.0 [8.0 – 13.0]	9.0 [8.0 – 11.0]	10 [8.0 – 12.0]
Mechanical ventilation — no. (%)	68 (58.6)	23 (74.2)	20 (62.5)	70 (63.1)
Vasopressor/inotropic therapy use — no. (%)	103 (88.8)	28 (90.3)	30 (93.8)	102 (91.9)
Vital signs				
Heart rate, median [IQR] — beats/min	98.0 [85.0 – 111.0]	93.0 [77.0 – 115.0]	90.0 [75.5 – 107.5]	95.5 [81.0 – 110.0]
Systolic blood pressure, median [IQR] — mm Hg	112.0 [101.5 – 131.5]	108.0 [100.0 – 119.0]	118.5 [96.0 – 131.0]	110.0 [98.0 – 123.0]
Diastolic blood pressure, median [IQR] — mm Hg	56.5 [51.0 – 63.5]	54.0 [49.0 – 62.0]	58.0 [54.0 – 62.0]	56.0 [50.0 – 62.0]
Body temperature — no. (%)				
<36°C	11 (9.5)	4 (12.9)	5 (15.6)	11 (9.9)
≥36°C to ≤38°C	76 (65.5)	20 (64.5)	22 (68.8)	79 (71.2)
>38°C	27 (23.3)	7 (22.6)	5 (15.6)	18 (16.2)
Renal function				
eGFR, median [IQR] — ml/min	37.5 [23.9 – 50.8]	27.2 [20.0 – 42.4]	25.6 [20.4 – 40.7]	29.7 [20.5 – 46.5]
ECC, median [IQR; no.] — ml/min				
Day 0	31.8 [14.7 – 62.5; 49]	14.4 [8.8 – 58.0; 10]	26.0 [5.4 – 30.6; 12]	24.1 [10.5 – 54.9; 46]
Day 1	35.9 [12.2 – 82.9; 103]	28.3 [4.3 – 64.4; 30]	25.2 [9.4 – 61.0; 31]	26.0 [8.8 – 59.5; 102]
AKI stage — no. (%)				
Stage 1	91 (78.4)	22 (71.0)	23 (71.9)	81 (73.0)
Stage 2	16 (13.8)	5 (16.1)	5 (15.6)	17 (15.3)
Stage 3	5 (4.3)	4 (12.9)	4 (12.5)	11 (9.9)
Urine output, median [IQR] — ml/h	60.0 [21.8 – 99.3]	50.0 [21.7 – 101.7]	27.4 [16.4 – 50.0]	39.1 [10.9 – 85.7]
Serum creatinine, median [IQR] — mg/dl	1.8 [1.3 – 2.4]	2.3 [1.5 – 2.8]	1.9 [1.5 – 2.8]	2.0 [1.4 – 2.8]

FIG. 5

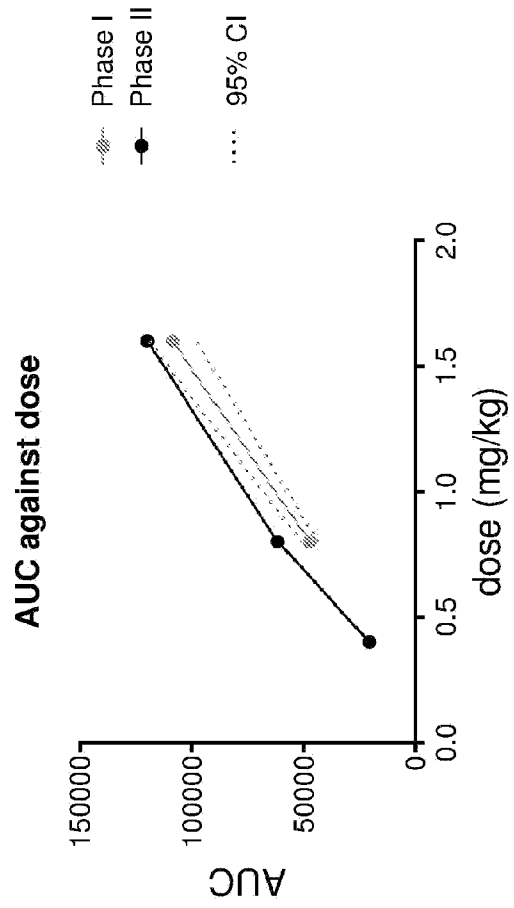


FIG. 6

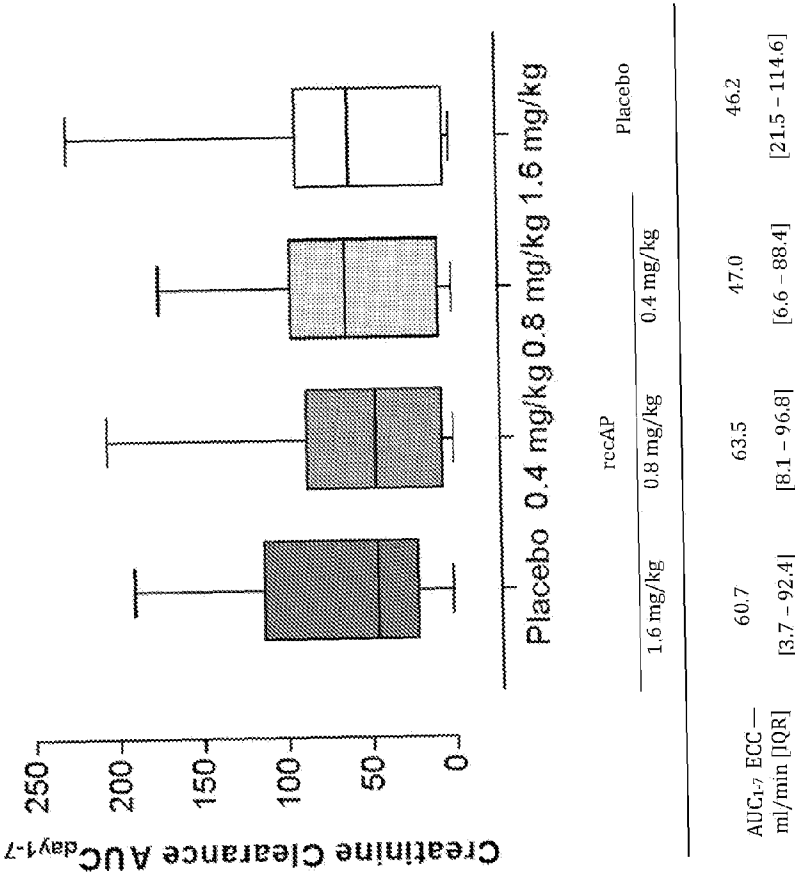


FIG. 7

Primary and Secondary Endpoints

Endpoint	Mean difference		P-value
	recAP 1.6 mg/kg	Placebo	(Lower; Upper limit 95% CI)
Primary endpoint			
Area under the time-corrected ECC curve (AUC ₀₋₇), median [IQR] — ml/min			
Part 1	60.7 [3.7-92.4]	46.2 [21.5-114.6]	-16.5 ^{ss} (-62.6; 29.5)
Part 2	51.8 [17.6-94.6]	44.5 [17.7-108.5]	-4.7 ^{ss} (-23.1; 13.8)
Part 1 + Part 2	55.1 [15.0-93.9]	45.6 [17.7-112.4]	-7.9 ^{ss} (-7.4; 23.0)
Secondary endpoints			
RRT incidence [§] (days 1-28) — %	36.0	29.3	1.4 ⁺⁺ (0.8; 2.4)
RRT-free days (days 1-28), median [IQR] — days	28 [16-28]	28 [16-28]	0.7 ^{ss} (1.8; 3.2)
Renal function, median [IQR] — ml/min			
Day 28 (ECC/cGFR)	88.0 [61.9 - 107.5]	82.6 [53.8 - 104.8]	-3.9 ^{ss} (-23.7; 15.9)
Day 60 (eGFR)	90.7 [75.8 - 108.9]	88.6 [74.1 - 104.0]	-1.9 ^{ss} (-10.4; 6.6)
Day 90 (cGFR)	91.1 [79.7 - 107.8]	88.6 [72.1 - 101.3]	-2.0 ^{ss} (-10.2; 6.3)
Composite endpoints (MAKE)— no. (%)			
MAKE 28*	42.3 (47)	41.4 (48)	1.0 ⁺⁺ (0.6; 1.6)
MAKE 60†	27.0 (30)	39.7 (46)	1.8 ⁺⁺ (1.0; 3.1)
MAKE 90‡	26.1 (29)	39.7 (46)	1.9 ⁺⁺ (1.1; 3.3)
Non-renal clinical endpoints			
Ventilator-free days (days 1-28), median [IQR] — days	17 [1 - 23]	14 [1 - 22]	1.3 ^{ss} (-1.7; 4.3)
Shock-free days (days 1-28), median [IQR] — days	23 [13 - 25]	22 [1 - 25]	1.8 ^{ss} (-1.0; 4.7)
ICU length of stay (days 1-90), median [IQR] — days	13 [7 - 28]	14 [6 - 28]	1.0 ⁺⁺ (-0.8; 1.3)
Hospital length of stay (days 1-90), median [IQR] — days	28 [22-28]	28 [24-28]	0.9 ⁺⁺ (-0.8; 1.3)
All-cause mortality at day 28 — no. (%)	16 (14.4)	31 (26.7)	12.3 (1.9 - 22.7)

FIG. 8

FIG. 9A Endogenous Creatinine Clearance

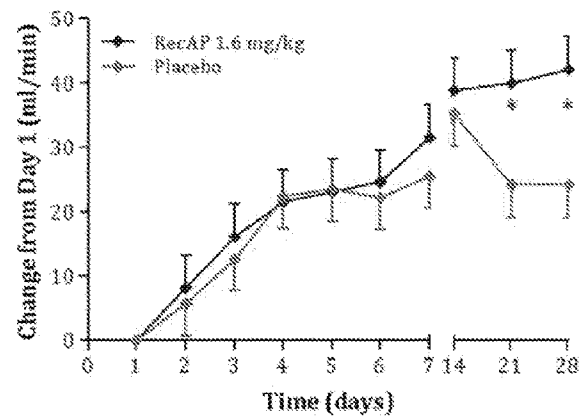
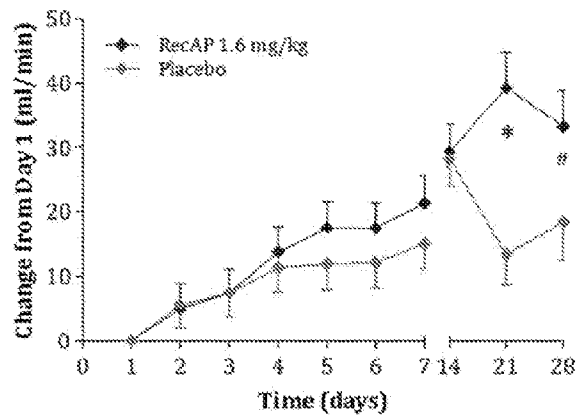


FIG. 9B BUN Clearance



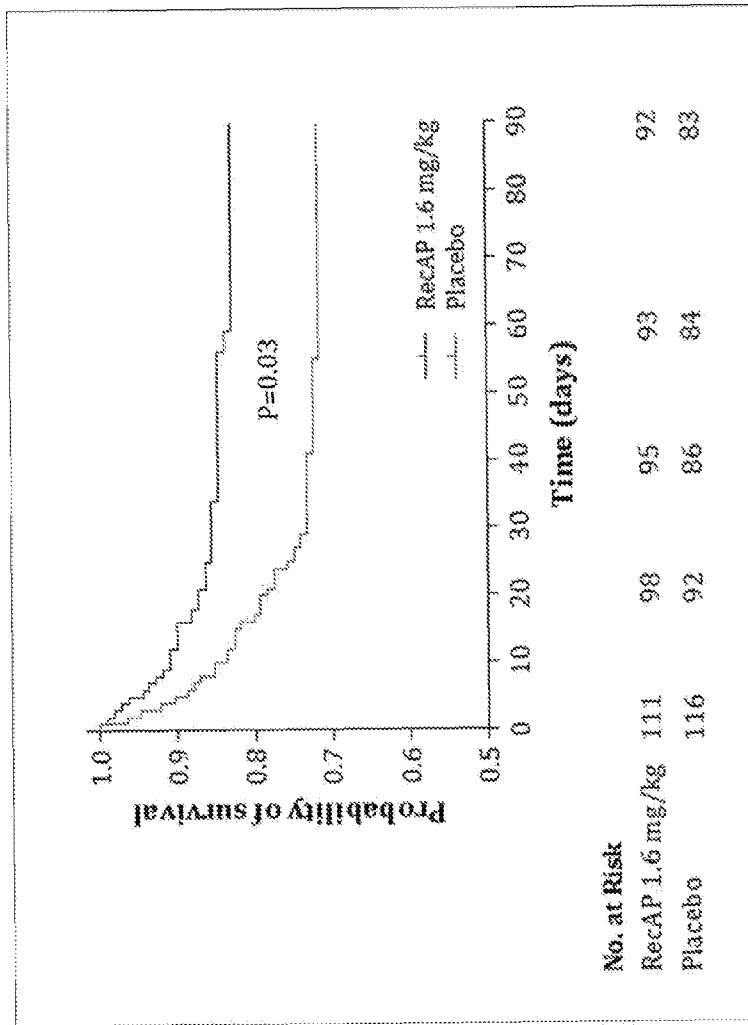


FIG. 10

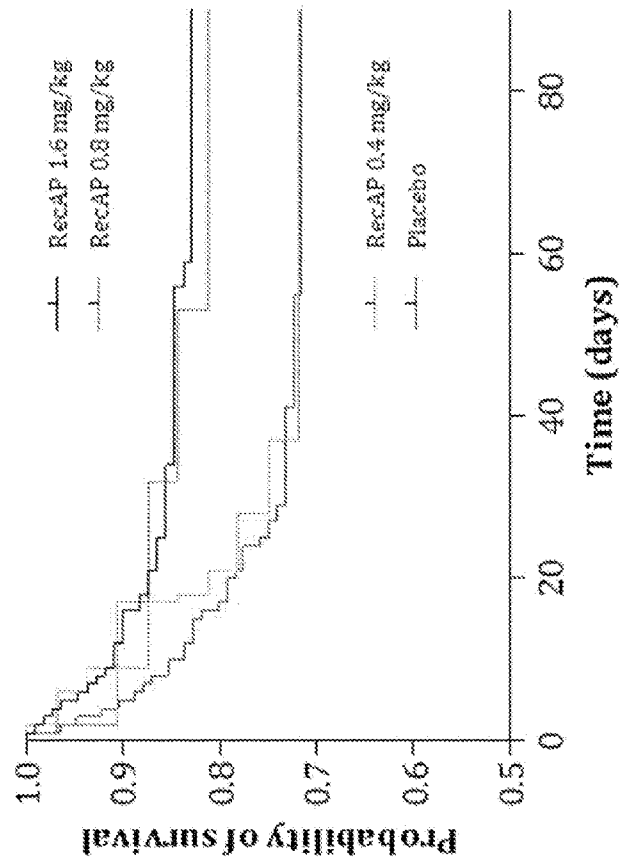


FIG. 11

PRIMARY AND SECONDARY ENDPOINTS FOR ALL GROUPS

Endpoint	recAP		Placebo (n=116)
	1.6 mg/kg (n=111)	0.8 mg/kg (n=32)	0.4 mg/kg (n=31)
Primary endpoint			
Area under the time-corrected ECC curve (AUC ₀₋₇), median [IQR; no.] — ml/min			
Part I	60.72 [3.67 – 92.41; 28] 51.76 [17.61 – 94.58; 80] 55.06 [15.01 – 93.88; 108]	63.54 [8.07 – 96.77; 32] NA 63.54 [8.07 – 96.77; 32]	46.95 [6.58 – 88.40; 30] NA 46.95 [6.58 – 88.40; 30]
Part II			46.16 [21.49 – 114.55; 29] 44.51 [17.71 – 108.51; 77] 45.58 [17.71 – 112.38; 106]
Secondary endpoints			
RRT incidence§ (day 1–28) — %	36.0	21.9	29.3
RRT-free days (day 1–28), median [IQR; no.] — days	28.0 [16.0 – 28.0; 111]	28.0 [21.0 – 28.0; 32]	28.0 [16.0 – 28.0; 116]
Renal function , median [IQR; no.] — ml/min			
Day 28 (ECC/eGFR)	88.0 [61.9 – 107.5; 73] 90.7 [75.8 – 108.9; 92] 91.1 [79.7 – 107.8; 92]	84.96 [62.6 – 119.4; 21] 87.14 [64.8 – 107.8; 26] 90.03 [66.2 – 105.5; 26]	89.63 [47.8 – 113.2; 18] 92.07 [69.0–99.5; 22] 89.46 [74.3 – 97.2; 22]
Day 60 (eGFR)			
Day 90 (eGFR)			
Composite endpoints (MAKE*) — no.(%)	47 (42.3) 30 (27.0) 29 (26.1)	9 (28.1) 11 (34.4) 10 (31.3)	15 (48.4) 13 (41.9) 13 (41.9)
MAKE 28*			48 (41.4)
MAKE 60†			46 (39.7)
MAKE 90‡			46 (39.7)
Non-renal clinical endpoints			
Ventilator-free days (days 1–28), median [IQR; no.]	17.0 [1.0– 23.0; 92]	20.0 [3.0 – 25.0; 27]	12.0 [2.0 – 22.0; 26]
Shock-free days (days 1–28), median [IQR; no.]	23.0 [13.0 – 25.0; 101]	23.0 [15.0 – 26.0; 30]	18.5 [4.0 – 25.5; 28]
ICU length of stay (days 1–90), median [IQR; no.] — days	13.0 [7.0 – 28.0; 109]	11.0 [5.5 – 28.0; 32]	16.0 [6 – 28; 31]
Hospital length of stay (days 1–90), median [IQR; no.] — days	28.0 [22.0–28.0; 109]	28.0 [14.0–28.5; 32]	28.0 [21.0 – 28.0; 31]
All-cause mortality at day 28 — no.(%)	16 (14.4)	4 (12.5)	8 (25.8)
			31 (26.7)

AUC1–7 denotes area under the endogenous creatinine clearance (ECC) curve from day 1 through day 7, divided by 7 to provide a time-corrected clearance in ml/min. CI indicates confidence interval. CKD-EPI Chronic Kidney Disease Epidemiology Collaboration. ECC endogenous creatinine clearance, eGFR estimated glomerular filtration rate, ICU intensive care unit, IQR interquartile range, MAKE major adverse kidney events, RRT renal-replacement therapy, and SOFA Sequential Organ Failure Assessment.

§ RRT incidence indicates the percentage of patients that needed RRT following randomisation.

|| With last observation carried forward.

* MAKE 28: received RRT before or on day 28 or died before or on day 28. Proportion that met at least one of the criteria.

† MAKE 60: eGFR <60 ml/min at day 60 estimated by the CKD-EPI formula based on serum creatinine or required chronic RRT, before or on day 60 or died before or on day 60. Proportion that met at least one of the criteria.

‡ MAKE 90: eGFR <60 ml/min at day 90 estimated by the CKD-EPI formula based on serum creatinine or required chronic RRT, before or on day 90 or died before or on day 90 or hospitalization for new episode of AKI before or on day 90 or died before or on day 90. Proportion that met at least one of the criteria.

FIG. 12

Quality of Life (QoL) measured by EuroQol5D (EQ5D) score at ICU discharge and Day 90

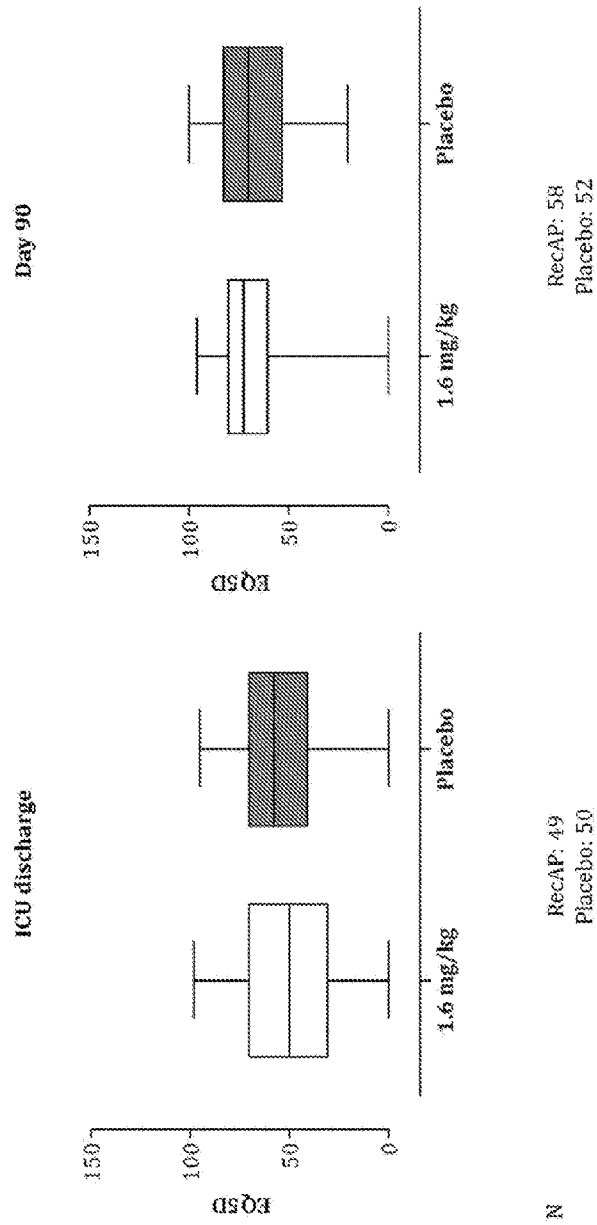


FIG. 13A

Lung parameters, depicted as medians [IQR]

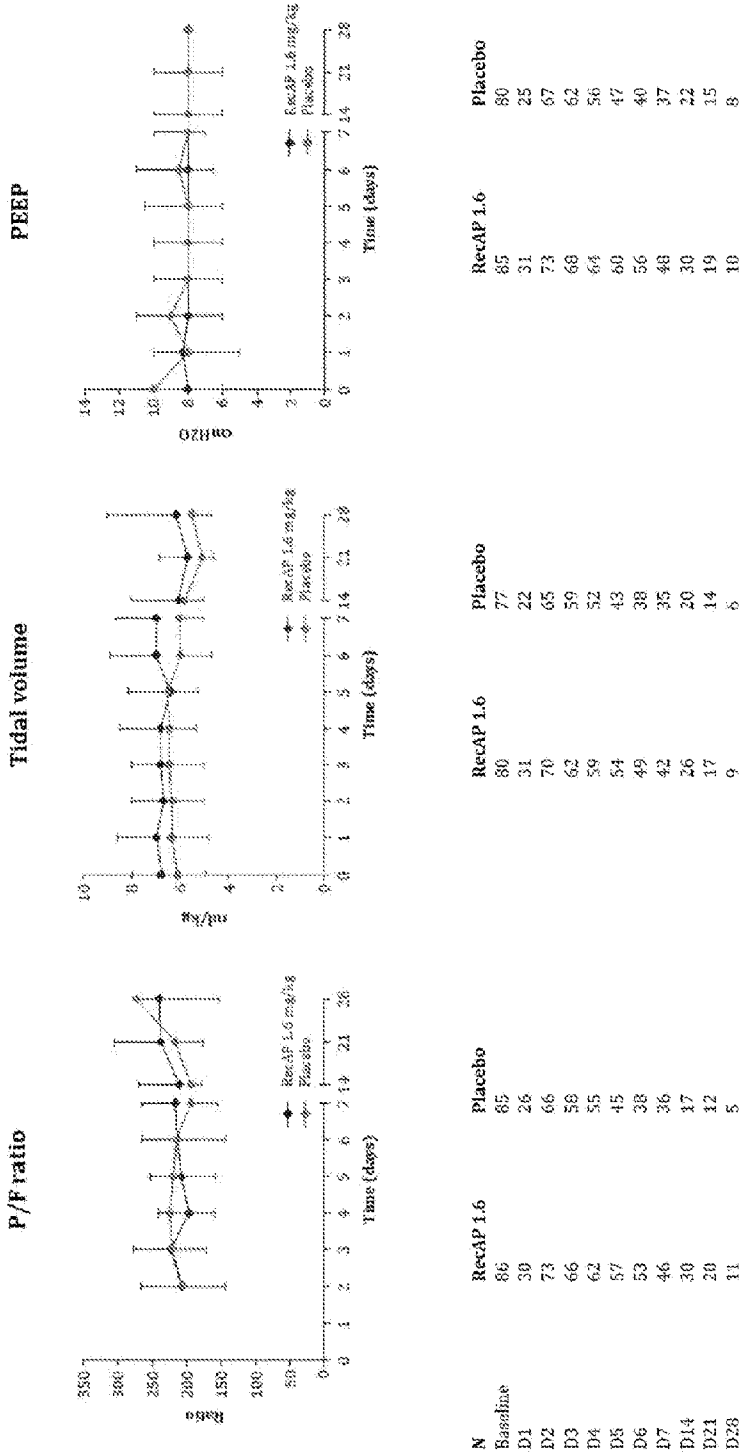


FIG. 13B

Liver parameters, depicted as medians [IQR]

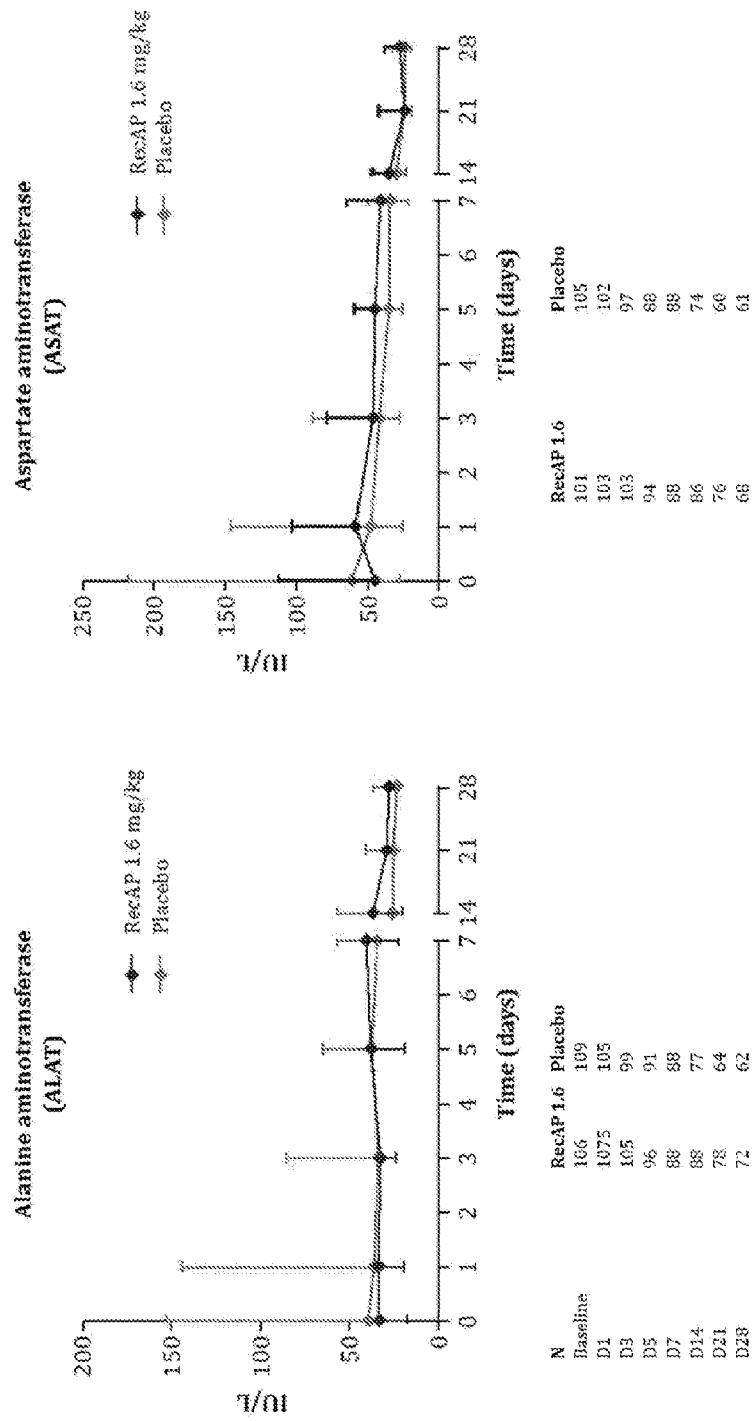


FIG. 13C

Liver parameters, depicted as medians [IQR]

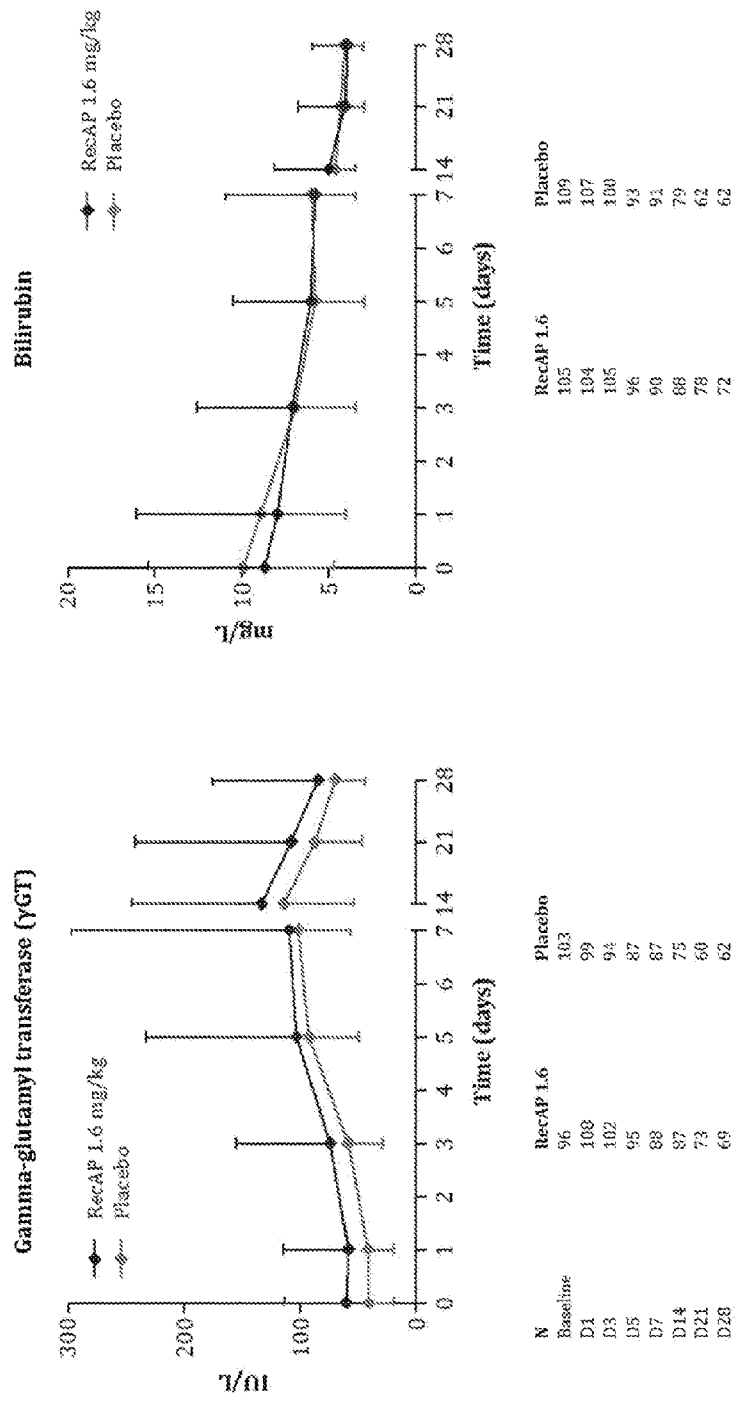


FIG. 13D

Liver parameters, depicted as medians [IQR]

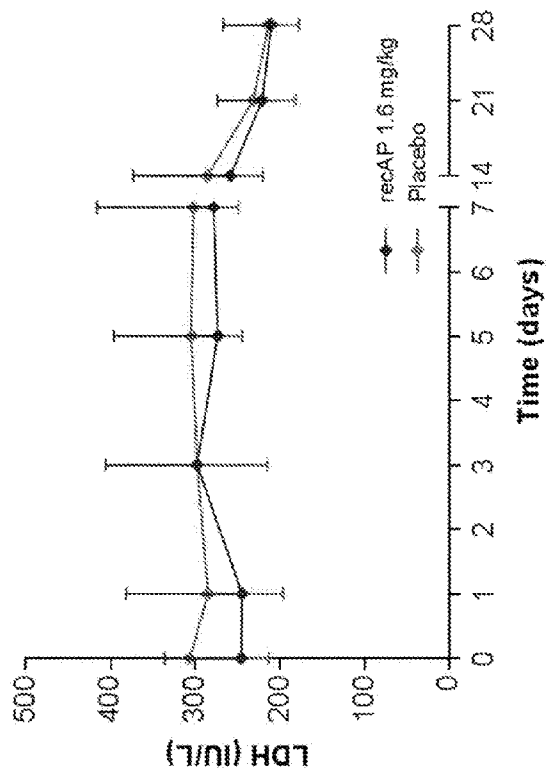


FIG. 13E

N	RecAP	Placebo
Baseline	95	97
D1	100	92
D3	97	87
D5	89	81
D7	82	84
D14	83	75
D21	72	59
D28	66	59

Parameter	Comparison	HR	lower CL	upper CL	P-value
Treatment arm	recAP 1.6 mg/kg vs placebo	0.50	0.28	0.899	0.020
SOFA Resp	{SOFA Resp + 1} vs SOFA Resp	1.68	1.22	2.31	0.001
SOFA CNS	{SOFA CNS + 1} vs SOFA CNS	1.17	1.00	1.39	0.057
SOFA Cardio	{SOFA Cardio + 1} vs SOFA Cardio	1.52	1.01	2.28	0.044
SOFA Liver	{SOFA Liver + 1} vs SOFA Liver	1.08	0.79	1.47	0.636
SOFA Coagulation	{SOFA Coag + 1} vs SOFA Coag	1.37	1.03	1.81	0.030
SOFA Renal	{SOFA Renal + 1} vs SOFA Renal	1.15	0.88	1.51	0.306

CL denotes confidence limit, and HR hazard ratio

- SOFA Resp: Respiratory system
- SOFA CNS: Central Nervous system
- SOFA Cardio: Cardiovascular system
- SOFA Liver: Liver
- SOFA Coag: Coagulation
- SOFA Renal: Renal

"SOFA Resp+1" indicates the increase in hazard ratio associated with an increase in the SOFA subgroup Respiratory of 1 point.

FIG. 14

C-reactive protein (CRP) concentrations (mg/L), depicted as medians [IQR]

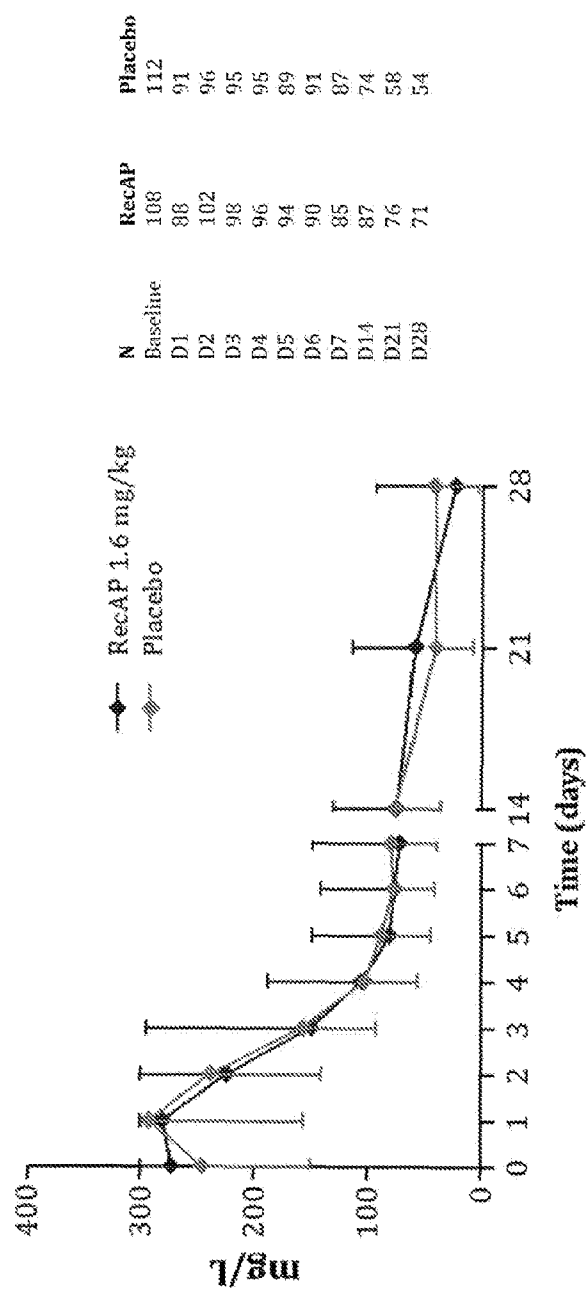


FIG. 15A

Interleukin 6 (IL-6) concentrations (pg/ml), depicted as medians [IQR]

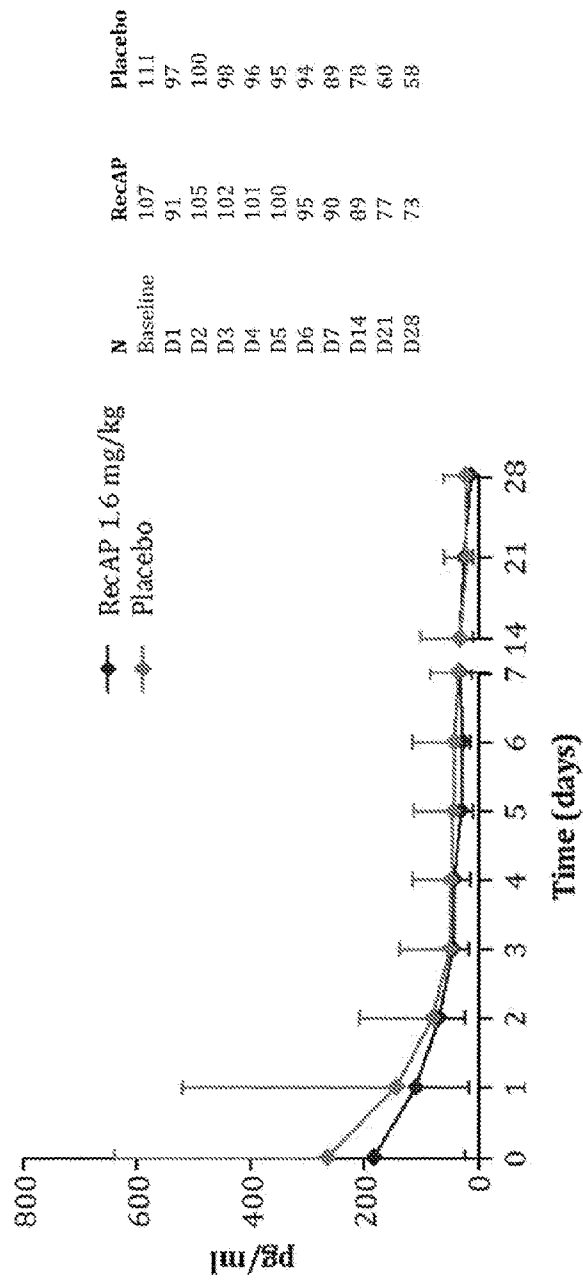


FIG. 15B

Interleukin 18 (IL-18) concentrations (pg/ml), depicted as medians [IQR]

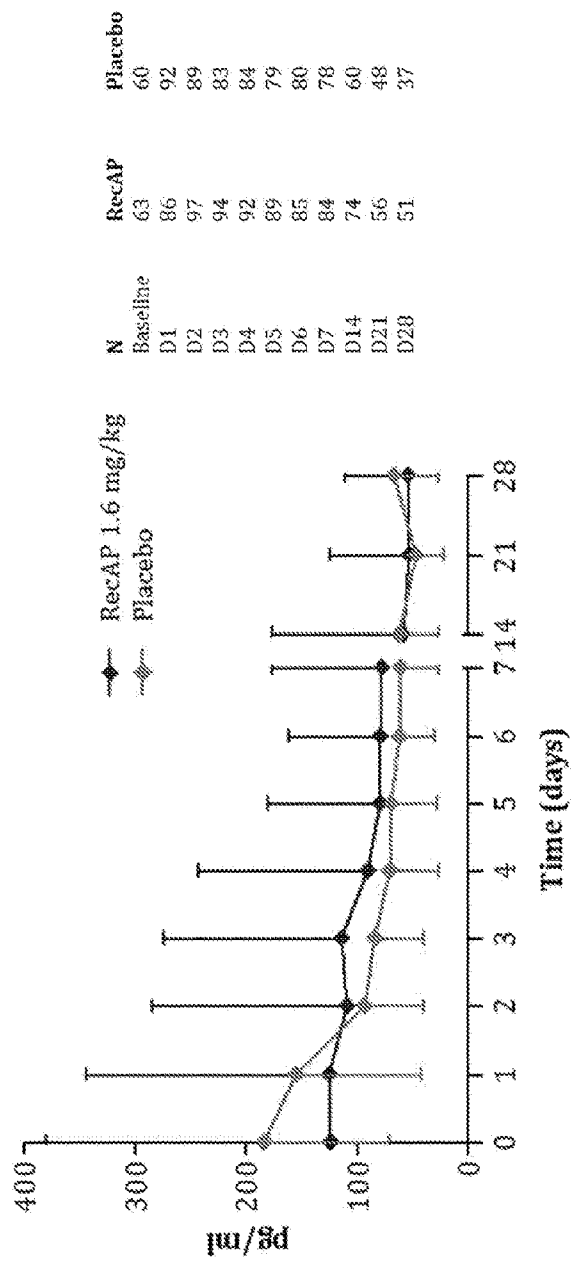


FIG. 15C

Lipopolysaccharide binding protein (LBP) concentrations ($\mu\text{g}/\text{ml}$), depicted as medians [IQR]

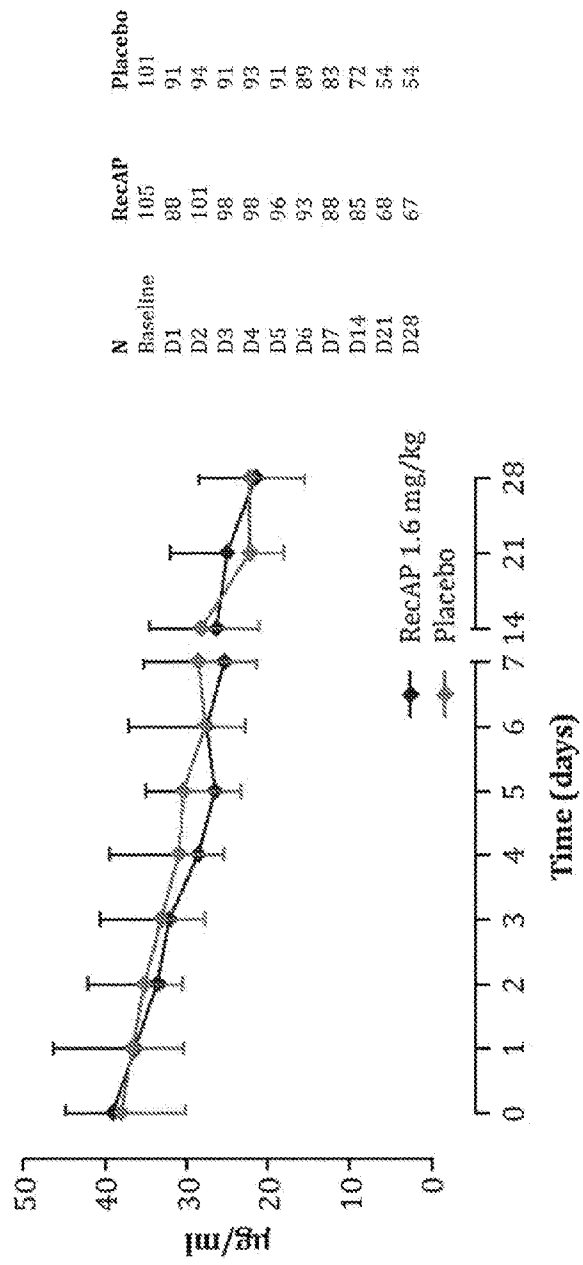


FIG. 15D

Kidney Injury Molecule 1 (KIM-1) concentrations (pg/ml), depicted as medians [IQR]

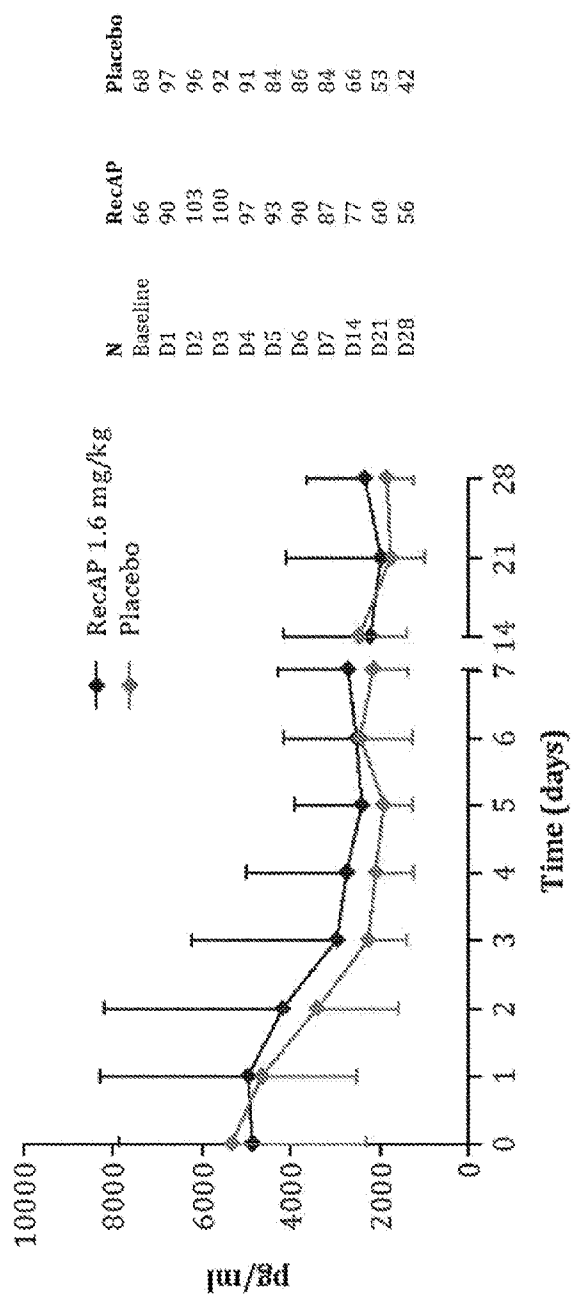


FIG. 15E

Influence of duration between first diagnosis of AKI and start of study drug infusion (hours) on primary endpoint ECC

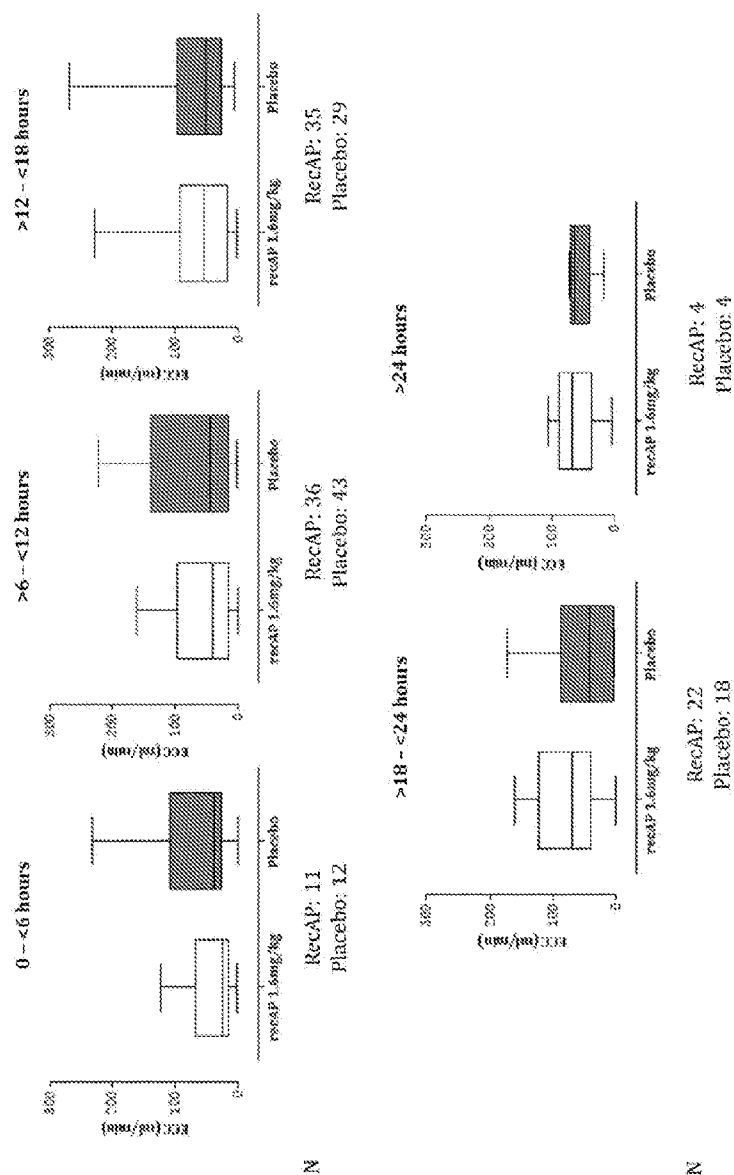


FIG. 15F

Influence of baseline disease severity by APACHE II score on primary endpoint ECC

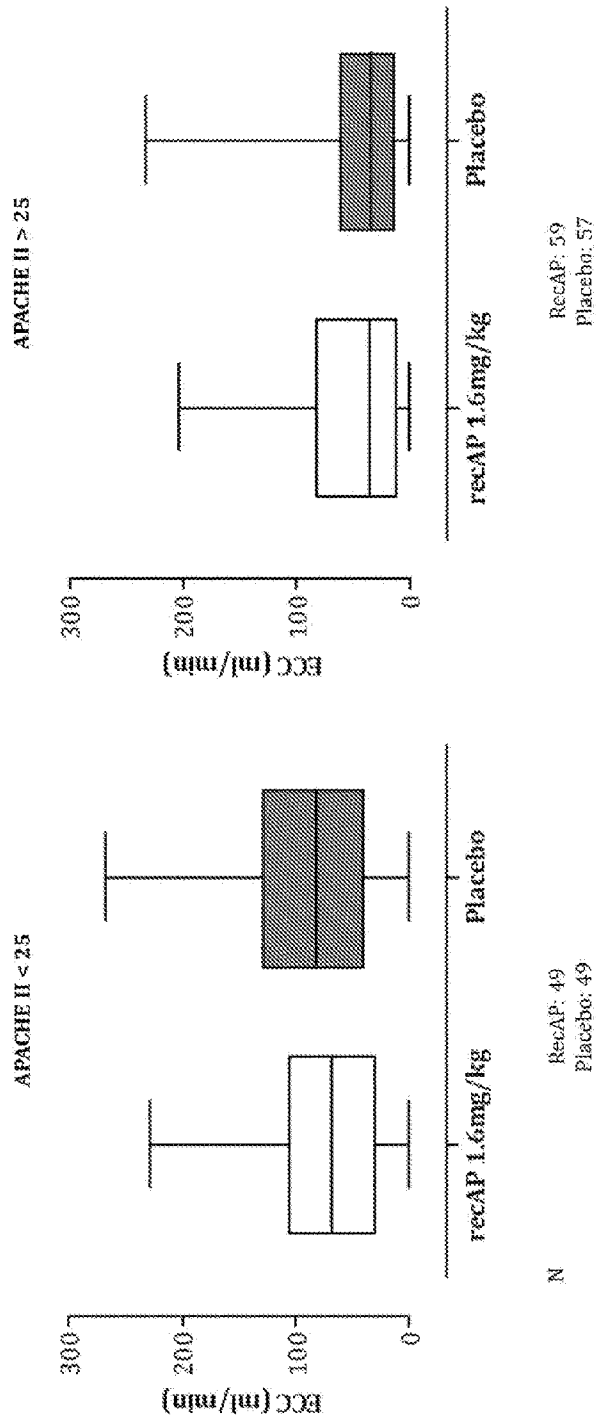


FIG. 15G

Influence of inflammation as measured by baseline C-reactive protein (CRP) concentrations (mg/L) on primary endpoint ECC

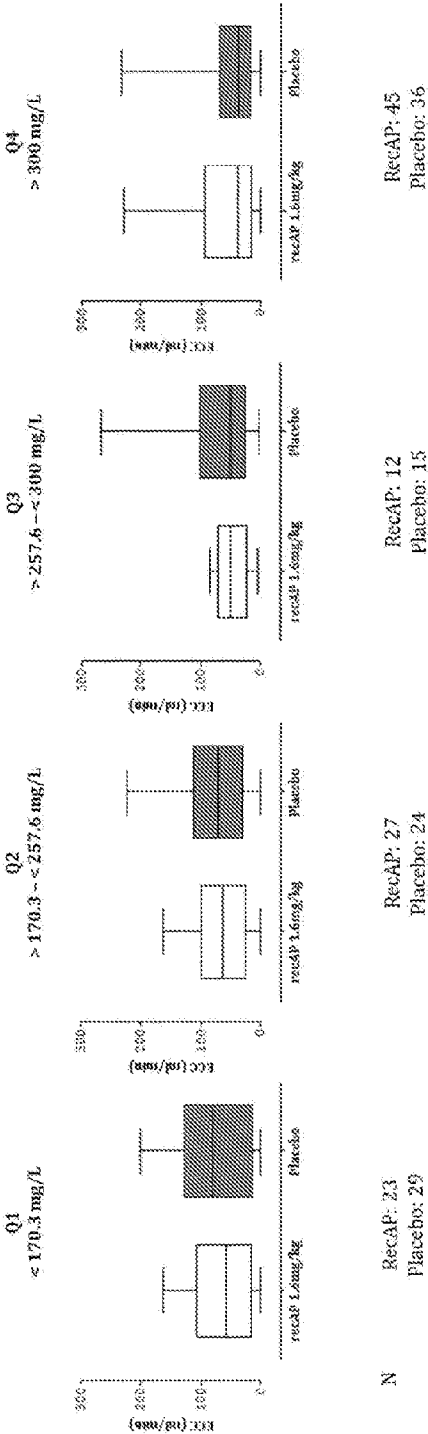


FIG. 15H

Influence of inflammation as measured by baseline interleukin 6 (IL-6) concentrations (pg/ml)
on primary endpoint ECC

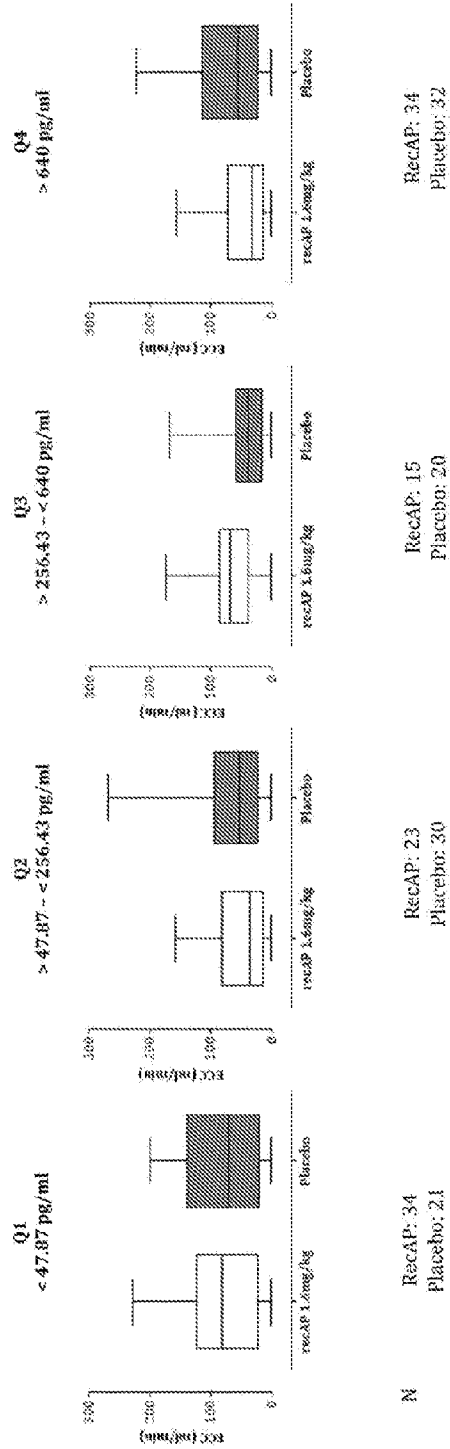


FIG. 15I

Influence of the fractional excretion of sodium (% of FeNa) at baseline on primary endpoint ECC

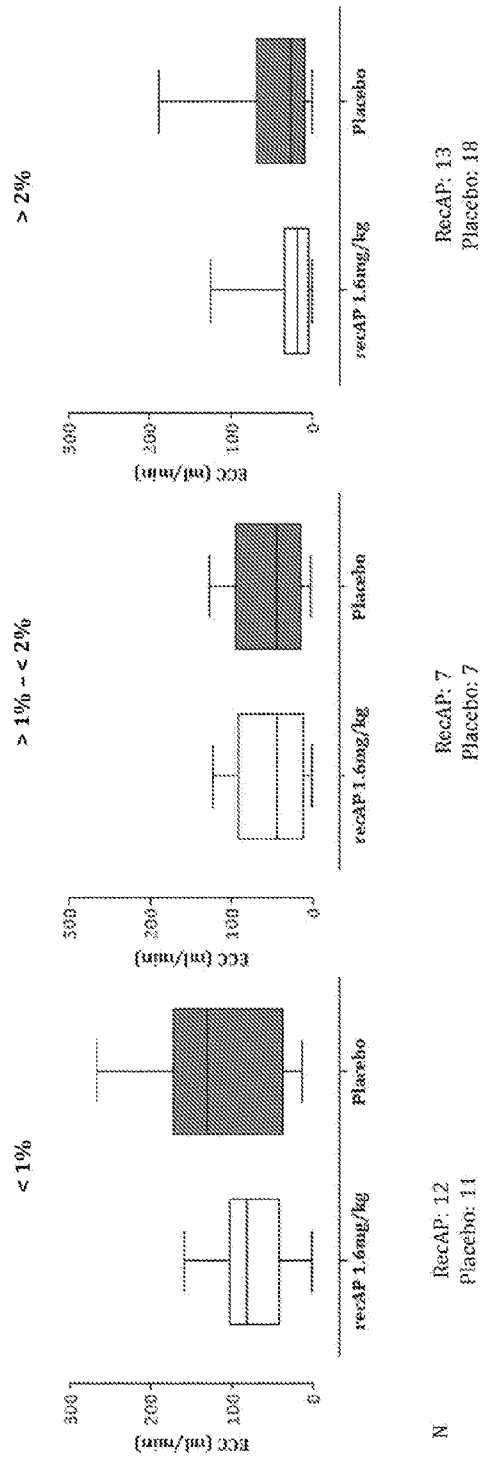


FIG. 15J

Influence of baseline Kidney Injury Molecule 1 (KIM-1) concentrations (pg/ml) on primary endpoint ECC

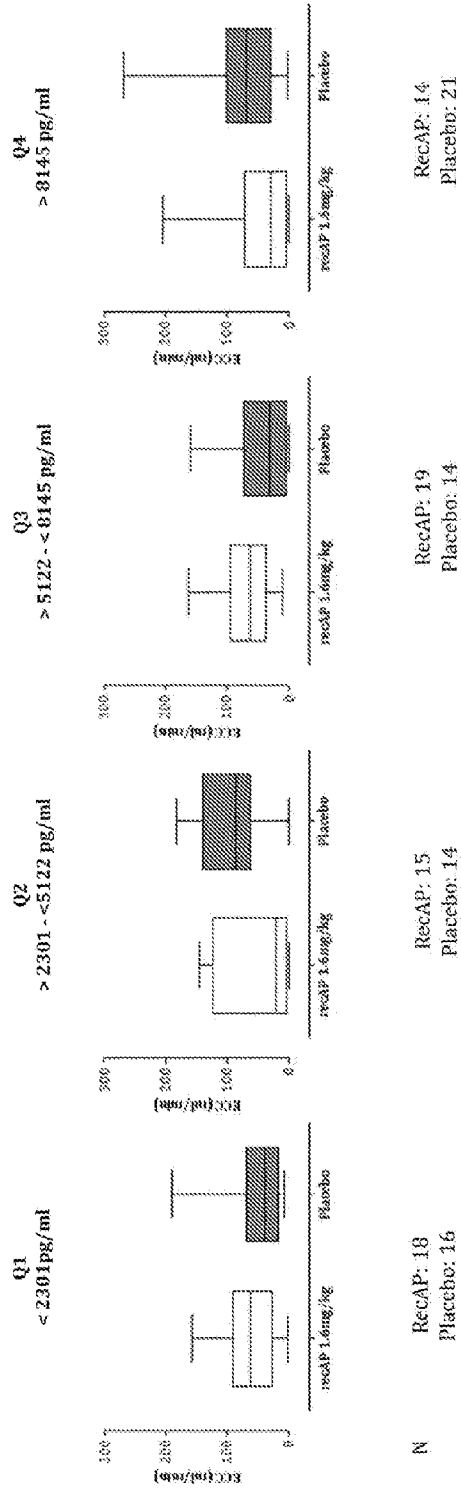


FIG. 15K

Difference in baseline kidney function on primary endpoint ECC, depicted as medians [IQR]

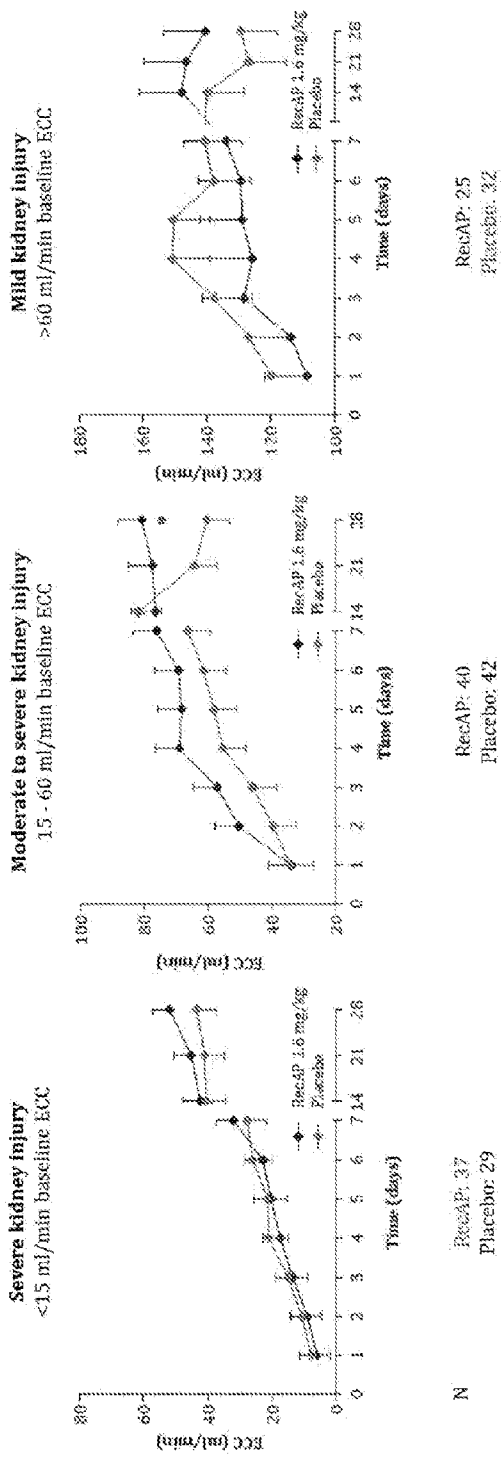


FIG. 15L

TREATMENT-EMERGENT (SERIOUS) ADVERSE EVENTS

Adverse event	Treatment emergent AEs				Treatment emergent SAEs			
	recAP				recAP			
	Placebo {N=112}	0.4 mg {N=38}	0.8 mg {N=35}	1.6 mg {N=109}	Placebo {N=112}	0.4 mg {N=36}	0.8 mg {N=35}	1.6 mg {N=109}
Total number of events	886	277	252	898	89	34	19	76
Number of patients with at least one treatment-emergent event	111 (99.1)	35 (92.1)	31 (88.6)	103 (94.5)	56 (59.0)	18 (47.4)	11 (31.4)	47 (43.1)
Gastrointestinal disorders	49 (43.8)	17 (44.7)	16 (45.7)	63 (57.6)	6 (5.3)	3 (7.9)	2 (5.7)	11 (10.1)
Infections and infestations	47 (42.0)	16 (42.1)	16 (45.7)	60 (55.0)	18 (16.1)	5 (13.2)	5 (14.3)	18 (15.5)
Metabolism and nutrition disorders	41 (36.6)	13 (34.2)	13 (37.1)	49 (45.0)	1 (0.9)	0	0	0
General disorders and administration site conditions	43 (38.4)	10 (26.3)	13 (37.1)	44 (40.4)	9 (8.0)	2 (5.3)	0	5 (4.6)
Cardiac disorders	44 (39.3)	11 (28.9)	12 (34.3)	43 (39.4)	11 (9.8)	0	3 (8.6)	9 (8.3)
Psychiatric disorders	39 (34.8)	12 (31.6)	13 (37.1)	42 (38.5)	0	0	0	1 (0.9)
Respiratory, thoracic and mediastinal disorders	52 (46.4)	14 (36.8)	14 (40.0)	40 (36.7)	14 (12.5)	6 (15.8)	3 (8.6)	9 (8.3)
Vascular disorders	40 (35.7)	12 (31.6)	9 (25.7)	36 (33.0)	7 (6.3)	1 (2.6)	0	5 (4.6)
Investigations	30 (26.0)	6 (21.1)	10 (28.6)	30 (27.5)	1 (0.9)	0	0	1 (0.9)
Blood and lymphatic system disorders	22 (19.6)	10 (26.3)	8 (22.9)	24 (22.0)	2 (1.8)	2 (5.3)	0	2 (1.8)
Skin and subcutaneous tissue disorders	17 (15.2)	7 (18.4)	5 (14.3)	22 (20.2)	0	0	0	1 (0.9)
Nervous system disorders	35 (31.4)	6 (15.8)	10 (28.6)	19 (17.4)	1 (0.9)	4 (10.5)	1 (2.9)	4 (3.7)
Renal and urinary disorders	12 (10.7)	6 (15.8)	2 (5.7)	16 (14.7)	2 (1.8)	1 (2.6)	0	1 (0.9)
Injury, poisoning and procedural complications	35 (31.4)	13 (34.2)	7 (20.0)	14 (12.8)	3 (2.7)	3 (7.9)	2 (5.7)	0
Musculoskeletal and connective tissue disorders	14 (12.5)	3 (7.9)	1 (2.9)	13 (11.9)	1 (0.9)	0	0	0
Hepatobiliary disorders	9 (8.0)	1 (2.6)	1 (2.9)	9 (8.3)	4 (5.3)	1 (2.6)	0	0
Endocrine disorders	4 (3.6)	1 (2.6)	1 (2.9)	3 (2.8)	0	0	0	0
Eye disorders	2 (1.8)	3 (7.9)	1 (2.9)	3 (2.8)	0	0	0	0
Ear and labyrinth disorders	1 (0.9)	0	0	2 (1.8)	0	0	0	1 (0.9)
Neoplasm benign, malignant and unspecified (incl cyst and polyps)	1 (0.9)	0	0	1 (0.9)	0	0	0	0
Congenital, familial and genetic disorders	0	0	0	1 (0.9)	0	0	0	0
Reproductive system and breast disorders	0	0	0	1 (0.9)	0	0	0	0

Data presented as no. (%)

AE denotes adverse event. SAE denotes serious adverse event.

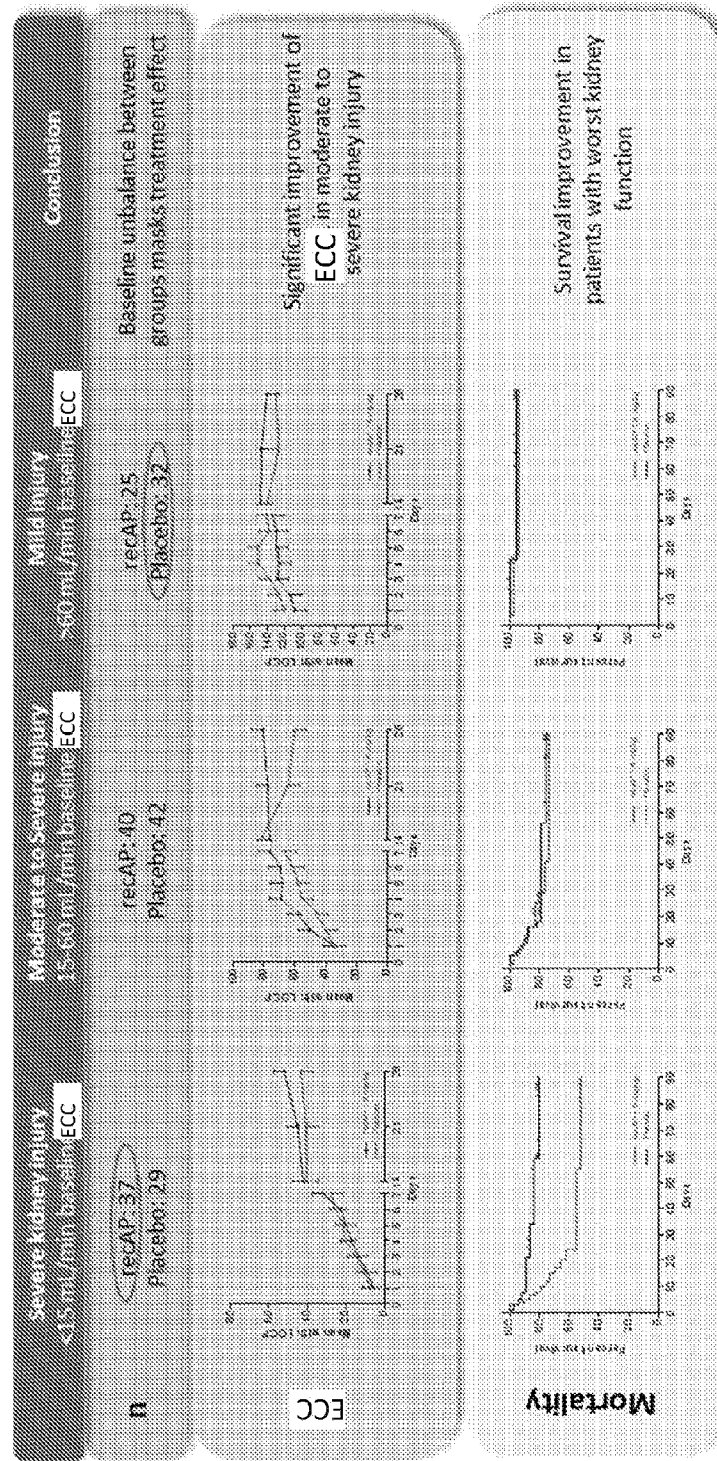
Treatment-emergent (serious) adverse events were coded according to the Medical Dictionary for Regulatory Activities. Patients were counted once for each event category, even if they had multiple events in that category. There were no significant differences at $P < 0.05$ between the groups in the percentage of patients with treatment-emergent (serious) adverse events.

FIG. 16

Subgroup analysis on baseline ECC

STOP-AKI

Baseline* significantly influences AUC_{1-7} and mortality



* Baseline ECC defined as the average between baseline and D1 ECC

FIG. 17

Cox model with ECC is highly significant
And comparable to Cox model with CKD

Table:

		Dependent variable:	
		OS	
		(1)	(2)
MeanBaseECC		-0.032*** (0.007)	
CKD	Severe-Mild		-0.751*** (0.274)
CKD	Normal		-2.435*** (0.602)
Observations		268	268
R ²		0.144	0.109
Log Likelihood		-298.534	-303.956
LR Test		41.786*** (df = 1)	30.941*** (df = 2)
Score (Logrank) Test		18.734*** (df = 1)	27.901*** (df = 2)

Note: ***p<0.01, **p<0.05, *p<0.1

FIG. 18

Impact of ECC on HR according to Cox model

By default linear on hazard scale

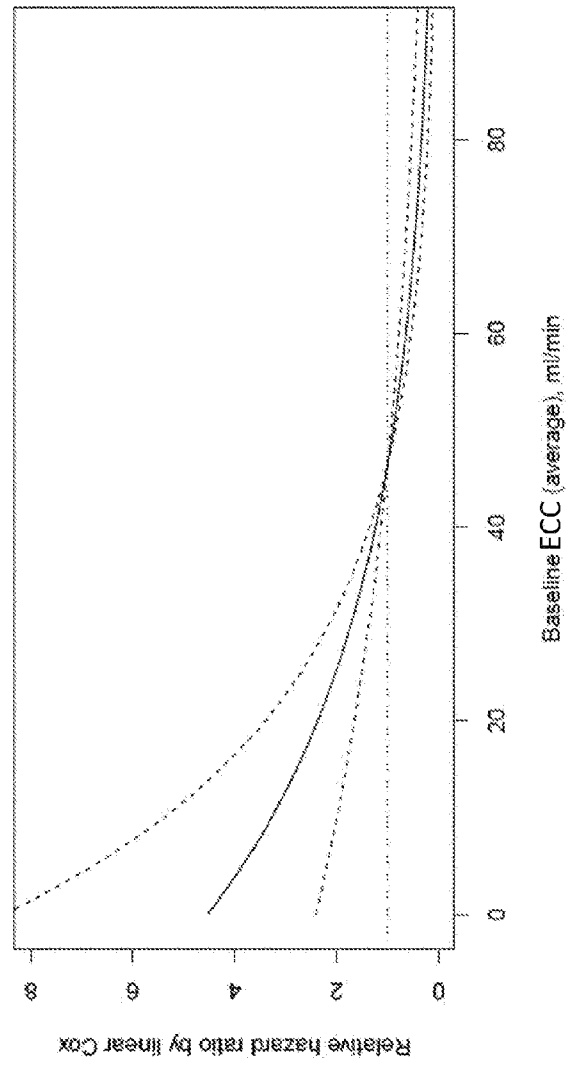


FIG. 19

Cox HR predictions for CKD group medians and cut-offs

CKD	median	n	HR	seHR
Kidney failure	5.46	87	3.79	28.6
Severe-Mild	31.2	109	1.64	10.6
Normal	97.3	72	0.192	35.3
critLow	15.0		2.78	21.9
critHigh	60.0		0.646	9.37

FIG. 20

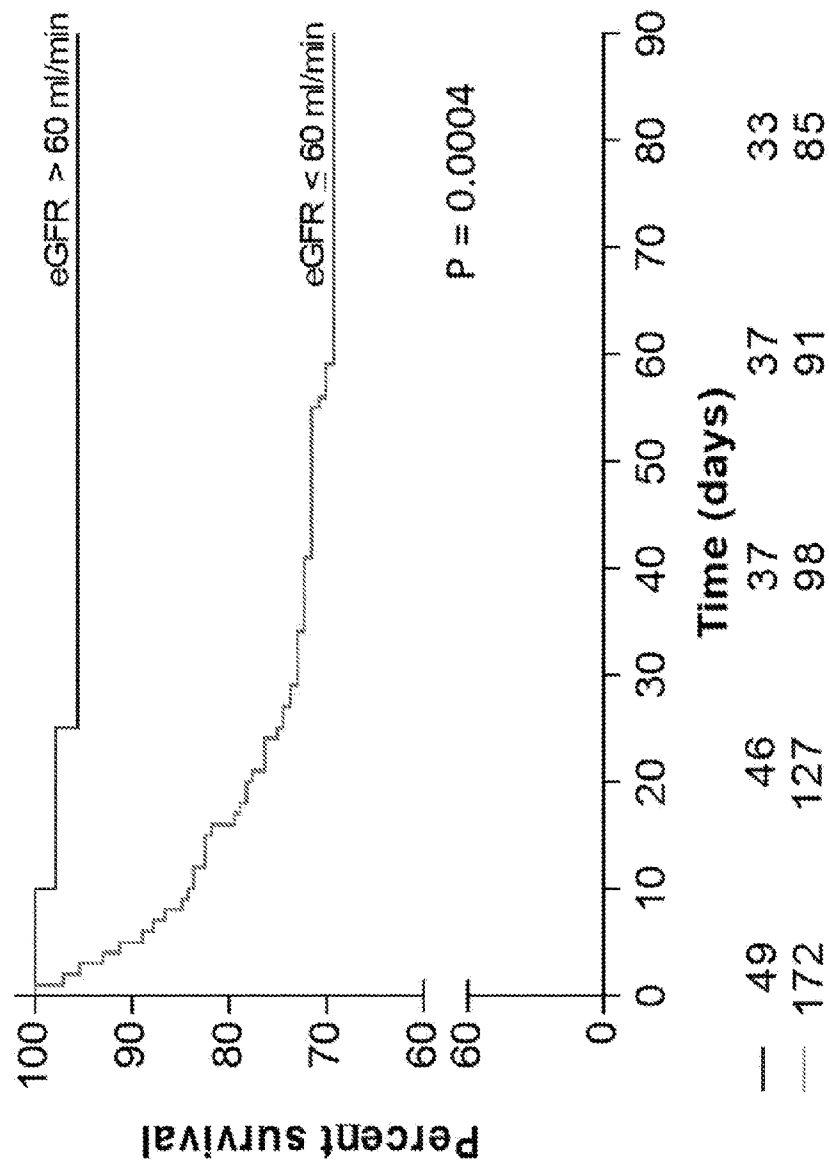


FIG. 21

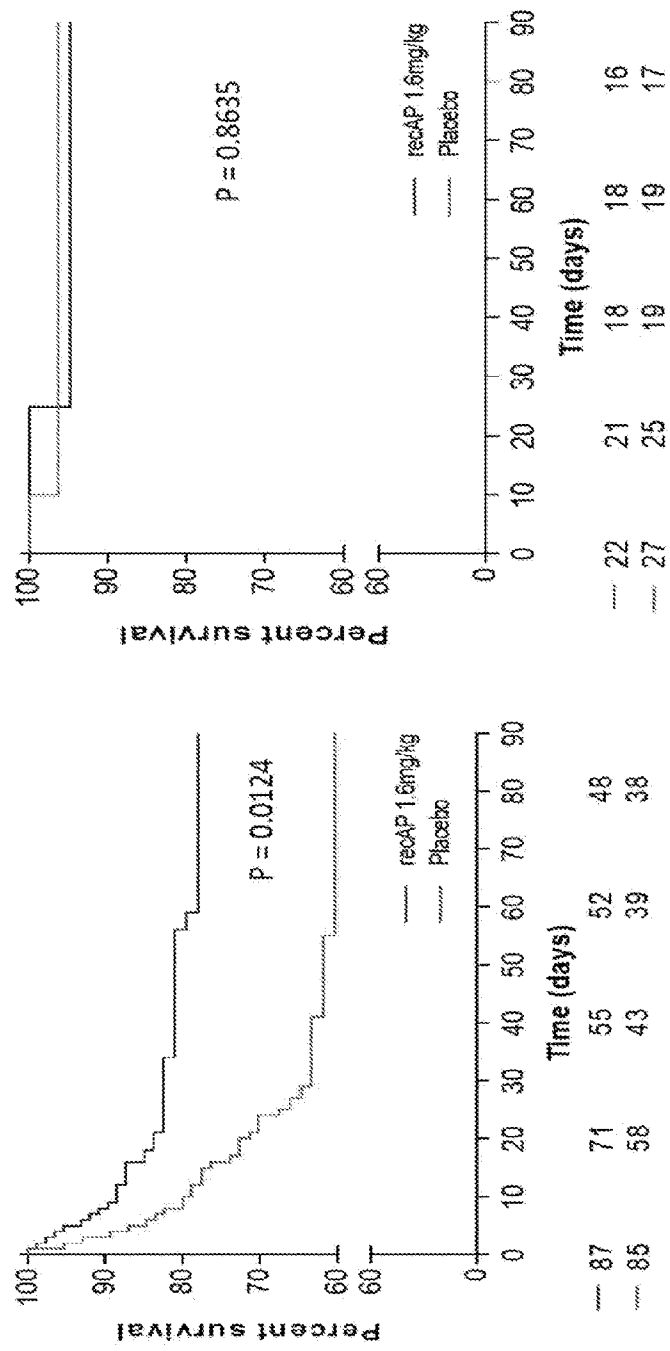


FIG. 22