PEER REVIEW HISTORY

BMJ Open publishes all reviews undertaken for accepted manuscripts. Reviewers are asked to complete a checklist review form (**http://bmjopen.bmj.com/site/about/resources/checklist.pdf**) and are provided with free text boxes to elaborate on their assessment. These free text comments are reproduced below.

ARTICLE DETAILS

TITLE (PROVISIONAL)	Study protocol for a multicenter randomized controlled trial: Safety, Tolerability, efficacy and quality of life Of a human recombinant alkaline Phosphatase in patients with sepsis-associated Acute Kidney Injury (STOP-AKI)
AUTHORS	Peters, Esther; Mehta, Ravindra; Murray, Patrick T; Hummel, Jurgen; Joannidis, Michael; Kellum, John; Arend, Jacques; PIckkers, Peter

VERSION 1 - REVIEW

REVIEWER	MACAULAY ONUIGBO MD MSc MBA MAYO CLINIC, ROCHESTER, MN, USA & MAYO CLINIC HEEALTH SYSTEM, EAU CLAIRE, WI, USA
REVIEW RETURNED	05-May-2016

GENERAL COMMENTS	Overall, a well designed and well written study of a very important clinical question. Page 12 - to add "angiotensin receptor blockers" after "angiotensin-converting enzyme inhibitors".

REVIEWER	Sukru Ulusoy Karadeniz Technical University, School of the Medicine, Department of the Nephrology President of the department of the nephrology Turkey
REVIEW RETURNED	11-May-2016

GENERAL COMMENTS	I have reviewed Peters et all's manuscript titled 'The STOP-AKI study: Sepsis Trial Of alkaline Phosphatase in Acute Kidney Injury. A safety, tolerability, efficacy and quality of life study of human recombinant alkaline phosphatase in the treatment of patients with sepsis-associated acute kidney injury: study protocol for a multicenter randomized controlled trial' This is the very interesting and exciting study. This study will clarify the treatment of the SA-AKI Aim of the study is very well descriptive. Study design/protocol and patient eligibility is suitable. I am looking forward the study results

REVIEWER	Mina Hur Department of Laboratory Medicine, Konkuk University School of Medicine, Seoul, Korea
REVIEW RETURNED	16-May-2016

GENERAL COMMENTS	This is a well-summarized study protocol for the clinical trial that would evaluate the safety and efficacy of recombinant alkaline phosphatase (rec-AP) in sepsis-associated acute kidney injury (SA-AKI). I would like to comment the following aspects. 1. Throughout the text, full names and abbreviations are mixed (for example, SA-AKI, rec-AP). Please, correct it. 2. In Introduction, sepsis definition was newly changed in 2016. Please, reflect this new definition in the manuscript as well as in the clinical trial. 3. Page 7, line 3-4: What are the related clinical parameters in detail? 4. Page 7, line 30-32: What are kidney function markers and biomarkers for tubular injury and systemic inflammation in detail? 5. Page 14, line 5-6: Biomarker endpoints should be described in detail, too. 6. Some references are described incompletely (#5, #28).

REVIEWER	Frederic T. (Josh) Billings, IV Assistant Professor of Anesthesiology and Medicine Vanderbilt University United States
REVIEW RETURNED	16-May-2016

GENERAL COMMENTS	Authors submit a report describing the design of the STOP-AKI study. This study, however, is well-underway, initiated 2014 and planned to conclude December, 2016. The overall design of the study is well-done, in particular the choice of the primary endpoint and the adaptive technique. The statistical analysis plan presented is lacking, which is somewhat concerning for a report on the methods of a clinical trial. Strengths: • Two-part adaptive trial provides opportunity to better investigate efficacy, dose-response, and any toxicity. • Multi-center trial • Attempt to assure septic patients are clinically managed with sepsis and AKI-related treatment guidelines. • Investigators are completing a Treatment study for sepsis induced AKI (the most common cause of AKI) rather than a Prevention study. A prevention study would require inclusion of many patients that may not develop AKI, that may not have end-organ damage, and that may therefore unnecessarily receive medication. Therefore, ultimately, AP may be selectively targeted for patients with a disease. • Use of CrCl endpoint to assess renal function post AKI. Well-conceived!

	Weaknesses: • Submission of this report follows completion of Part 1 (dose finding part of study) of trial rather than prior to initiating the trial. And since review of data for Part 1 was required to initiate Part 2 it should be made clear that this report was written after Part 1 of the study was completed and those data, including treatment assignments and outcomes, analyzed. • 50+ sites for 300 patients is average of 6 patients per site. Many sites will study even fewer patients. These sites may have poor per-protocol adherence to study intervention and primary outcome assessment. Consider reducing number of sites to achieve target enrollment. • Description and utility of the confirmative fluid-corrected serum creatinine measure in Table 1 is unclear. Does this require an additional serum creatinine measurement following diagnosis of AKI but before initiating treatment? • An example of the primary endpoint calculation (time-corrected 7-day creatinine clearance) is needed. Maybe in the form of a table with several mock patients or patients from Part 1 of the study showing the timing of all measurements used in the calculation (twice daily serum creatinines and the intermittent UOP volume?). • Baseline creatine assignment as the lowest value within 3 months of admission preselects for low outliers and biases towards increased AKI. Median creat in this period might be a better choice. AKI as an inclusion criterion rather than an outcome tempers this weakness. • CKD patients excluded. CKD patients are a group that most need renal therapy. Discussion of rational for this exclusion criterion (toxicity concern? Other?) is warranted. • The CrCl of a 6-hour period is assumed to reflect a 24h period although this very well may not be the case particularly during AKI (not steady state). A 6-hour period is reasonable due to staffing feasibility and daily assessments likely addresses lack of measurement between periods. The impact of using a 6-hour period should be mentioned. • Impact of censoring due to death or RRT on primary endpoint needs elaboration. • Secondary endpoint list is extensive and not well defined – non-specific. “liver enzymes”, “lung function”, “composite endpoints”… This list needs focus. It is also unclear if these markers are safety endpoints to evaluate potential toxicity or efficacy endpoints. If efficacy, discussion of adjustments for multiple comparisons is warranted. • The sample size calculation only mentions alpha, power, and sample size. Treatment effect and variability are required to calculate sample size for a continuous endpoint (such as AUC). These additional data are needed. • In the “Interim analysis” sections, the authors state, “the study may be terminated for futility if none of the three recAP doses in Part 1 show evidence of efficacy.” By what criteria? Evidence of efficacy with N=30 in each group is unlikely. Will this efficacy be evaluated based on clinical significance? • The specific statistics used to determine dose for Part 2 should be described. • Should there be a statistical penalty for combining the results of Part 1 and Part 2 since the results of Part 1 must be analyzed to determine Part 2 dose (alpha spending)? • “Where appropriate, predefined sensitivity analysis and exploratory analysis will be employed.” If these analyses are predefined, please

	define them. • Ethics section should be changed to past tense since IRB has already approved study at the time of this report (“….were approved by the IRB..) • More specifics on the role of the sponsor should be added, with relation to interpretation of analyses and drafting of the manuscript.

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1
Reviewer Name: MACAULAY ONUIGBO MD MSc MBA Institution and Country: MAYO CLINIC, ROCHESTER, MN, USA & MAYO CLINIC HEEALTH SYSTEM, EAU CLAIRE, WI, USA Competing Interests: None.

Overall, a well designed and well written study of a very important clinical question.

Page 12 - to add "angiotensin receptor blockers" after "angiotensin-converting enzyme inhibitors".

Answer: As requested by the reviewer, this is now added on page 12, section ‘Concomitant Medications’.

Reviewer: 2
Reviewer Name: Sukru Ulusoy
Institution and Country: Karadeniz Technical University, School of the Medicine, Department of the Nephrology, President of the department of the nephrology, Turkey Competing Interests: None

I have reviewed Peters et all’s manuscript titled ‘The STOP-AKI study: Sepsis Trial Of alkaline Phosphatase in Acute Kidney Injury. A safety, tolerability, efficacy and quality of life study of human recombinant alkaline phosphatase in the treatment of patients with sepsis-associated acute kidney injury: study protocol for a multicenter randomized controlled trial’

This is the very interesting and exciting study. This study will clarify the treatment of the SA-AKI Aim of the study is very well descriptive. Study design/protocol and patient eligibility is suitable.
I am looking forward the study results

Answer: The authors thank the reviewer for careful reading of our manuscript.

Reviewer: 3
Reviewer Name: Mina Hur
Institution and Country: Department of Laboratory Medicine, Konkuk University School of Medicine, Seoul, Korea
Competing Interests: None declared

This is a well-summarized study protocol for the clinical trial that would evaluate the safety and efficacy of recombinant alkaline phosphatase (rec-AP) in sepsis-associated acute kidney injury (SA-AKI). I would like to comment the following aspects.

1. Throughout the text, full names and abbreviations are mixed (for example, SA-AKI, rec-AP). Please, correct it.

Answer: As requested, this has been corrected throughout the manuscript.

2. In Introduction, sepsis definition was newly changed in 2016. Please, reflect this new definition in the manuscript as well as in the clinical trial.

Answer: We now reflect on the newly changed sepsis definition in the Discussion, on page 20-21.

3. Page 7, line 3-4: What are the related clinical parameters in detail?

Answer: These parameters are liver enzymes, lung function, mechanical ventilation over 28 days, shock over 28 days, SOFA scores during ICU stay, and mortality over 90 days) These parameters are now described in more detail at page 13-14, section 'other secondary endpoints'.

4. Page 7, line 30-32: What are kidney function markers and biomarkers for tubular injury and systemic inflammation in detail?

Answer: Kidney function markers are assessed by urine and serum creatinine, BUN, sodium, proteinuria, fractional excretion of sodium and urea and urine volume, biomarkers for tubular injury are KIM-1, IL-18 and GST-α and biomarkers for systemic inflammation are IL-6, CRP and LBP. This is now described in more detail at page 13-14, section 'other secondary endpoints'.

5. Page 14, line 5-6: Biomarker endpoints should be described in detail, too.

Answer: These endpoints are now described in more detail at page 13-14, section 'other secondary endpoints'.

6. Some references are described incompletely (#5, #28).

Answer: We updated the incomplete references.

Reviewer: 4
Reviewer Name: Frederic T. (Josh) Billings, IV
Institution and Country: Assistant Professor of Anesthesiology and Medicine, Vanderbilt University, United States
Competing Interests: None declared

Authors submit a report describing the design of the STOP-AKI study. This study, however, is well-underway, initiated 2014 and planned to conclude December, 2016. The overall design of the study is well-done, in particular the choice of the primary endpoint and the adaptive technique. The statistical analysis plan presented is lacking, which is somewhat concerning for a report on the methods of a clinical trial.

Strengths:
• Two-part adaptive trial provides opportunity to better investigate efficacy, dose-response, and any toxicity.
• Multi-center trial
• Attempt to assure septic patients are clinically managed with sepsis and AKI-related treatment guidelines.
• Investigators are completing a Treatment study for sepsis induced AKI (the most common cause of AKI) rather than a Prevention study. A prevention study would require inclusion of many patients that may not develop AKI, that may not have end-organ damage, and that may therefore unnecessarily receive medication. Therefore, ultimately, AP may be selectively targeted for patients with a disease.
• Use of CrCl endpoint to assess renal function post AKI. Well-conceived!

Answer: We acknowledge the reviewer for carefully reading the manuscript and emphasising the strengths of the protocol.

Weaknesses:
• Submission of this report follows completion of Part 1 (dose finding part of study) of trial rather than prior to initiating the trial. And since review of data for Part 1 was required to initiate Part 2 it should be made clear that this report was written after Part 1 of the study was completed and those data, including treatment assignments and outcomes, analyzed.

Answer: Although submission follows completion of Part 1 of the trial, the manuscript was already completed prior to the interim analysis that took place 8th of April 2016. In addition, data of the interim-analyses have only been analyzed by the independent DMB, not by the authors nor the sponsor. This is now described more clearly in section 'Interim analysis' on page 15 and section 'Study period' on page 18.

• 50+ sites for 300 patients is average of 6 patients per site. Many sites will study even fewer patients. These sites may have poor per-protocol adherence to study intervention and primary outcome assessment. Consider reducing number of sites to achieve target enrollment.

Answer: We acknowledge that sites recruiting a low number of patients adherence is a limitation, that is shared by many other sepsis-trials. Importantly, so far, approximately 50% of the sites are actively recruiting and 50% of sites is dormant. Therefore, not recruiting sites will be closed. Evaluation of performance is happening on an individual and continuous basis. In principle, sites that have not recruited any patients for considerable time and without good reasons will be closed.

• Description and utility of the confirmative fluid-corrected serum creatinine measure in Table 1 is unclear. Does this require an additional serum creatinine measurement following diagnosis of AKI but before initiating treatment?

Answer: Indeed, following diagnosing AKI, continuing AKI has to be confirmed by a confirmative serum creatinine measure (corrected for fluid administration that is given in the mean time), defined as no decrease in serum creatinine ≥ 26.2 µmol/L (≥0.30 mg/dL). The reason for this is that resolving AKI patients should not be enrolled in this trial. These results have to be available preceding randomization and thus before initiating treatment, but still within the 24 h time-window, as treatment has to be administered within 24 h after diagnosing SA-AKI. This is now described more clearly in Table 1.

• An example of the primary endpoint calculation (time-corrected 7-day creatinine clearance) is needed. Maybe in the form of a table with several mock patients or patients from Part 1 of the study showing the timing of all measurements used in the calculation (twice daily serum creatinines and the intermittent UOP volume?).

Answer: To provide more details on the primary endpoint calculation, we now rephrased the section 'Primary endpoint' on page 12-13 and we added the formula to calculate the time-corrected 7-day creatinine clearance. We feel that this will provide the reader with sufficient information regarding the calculation of the primary endpoint.

• Baseline creatinine assignment as the lowest value within 3 months of admission preselects for low outliers and biases towards increased AKI. Median creatinine in this period might be a better choice. AKI as an inclusion criterion rather than an outcome tempers this weakness.

Answer: Indeed, a baseline creatinine as the lowest value within 3 months of admission may biases towards increased AKI. However, the aim of this study is to demonstrate whether recAP treatment results in improved creatinine clearance, and therefore, whether serum creatinine values return to their (lowest) baseline levels.

• CKD patients excluded. CKD patients are a group that most need renal therapy. Discussion of rational for this exclusion criterion (toxicity concern? Other?) is warranted.

Answer: The aim of this study is to investigate the efficacy of recAP to treat sepsis patients with AKI. As CKD is a disease with a distinct pathophysiology compared to AKI, inclusion of CKD patients is beyond the scope of this study. 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 were excluded. There is no toxicity concern, as previous studies have shown that adverse effects or toxicity was not observed in patients with impaired renal function or on dialysis. This is now described in the legend of Table 1.

• The CrCl of a 6-hour period is assumed to reflect a 24h period although this very well may not be the case particularly during AKI (not steady state). A 6-hour period is reasonable due to staffing feasibility and daily assessments likely addresses lack of measurement between periods. The impact of using a 6-hour period should be mentioned.

Answer: Indeed, 24h periods are the most optimal to determine creatinine clearance. However, previous publications show that 8-hour urine collection periods results in clearance values within 20% of the 24-hour clearances value (Baumann, Clin Pharm, 1987) and that 4-hour creatinine clearance measurements are superior to plasma creatinine for monitoring renal function in the critically ill (Pickering, Crit Care, 2012). A 6-hour period can be performed during 1 ICU nurse shift and is therefore most feasible. We now added the reference of Baumann to the section ‘Primary endpoint’, and mention the impact of a 6-hour period.

• Impact of censoring due to death or RRT on primary endpoint needs elaboration.

Answer: In case of death, missing clearance values will be imputed by ‘last observation carried forward’ (LOCF) method. When there are no preceding post-baseline measurements to use, the baseline measurement from Day 0 (prior to treatment) will be carried forward. When patients on RRT are oliguric, endogenous creatinine clearance can and will be calculated. If there is no diuresis, eGFR is zero. This is now described in more detail in section ‘Primary endpoint’ on page 12-13.

• Secondary endpoint list is extensive and not well defined – non-specific. “liver enzymes”, “lung function”, “composite endpoints”… This list needs focus. It is also unclear if these markers are safety endpoints to evaluate potential toxicity or efficacy endpoints. If efficacy, discussion of adjustments for multiple comparisons is warranted.

Answer: As requested by the reviewer, the section ‘Other secondary endpoints’ is now more detailed and better structured. As all analyses on the other secondary endpoints are for exploratory purposes only, no further multiplicity adjustments is required. This is now described in more detail in section ‘Statistical Analyses’.

• The sample size calculation only mentions alpha, power, and sample size. Treatment effect and variability are required to calculate sample size for a continuous endpoint (such as AUC). These additional data are needed.

Answer: The reviewer is correct, this information is now added to section ‘Sample Size Calculations’

page 14.

• In the “Interim analysis” sections, the authors state, “the study may be terminated for futility if none of the three recAP doses in Part 1 show evidence of efficacy.” By what criteria? Evidence of efficacy with N=30 in each group is unlikely. Will this efficacy be evaluated based on clinical significance?

Answer: At the interim analysis, the data will be reviewed to identify whether none of the recAP doses show evidence of efficacy. The results will be 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 comparisons to placebo. At the futility analysis, each recAP dose will 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. If none of the 3 recAP doses in Part 1 show evidence of efficacy (i.e. none of the doses have a one-sided, unadjusted p-value less than 0.8), the DMC will recommend further discussion with the sponsor and Steering Committee to determine whether the trial should be terminated for futility. This is now described more clearly at the section ‘Interim analysis’ page 15.

• The specific statistics used to determine dose for Part 2 should be described.

Answer: The specific statistics are now described in more detail in sections ‘Interim analysis’ and ‘Statistical analyses’.

• Should there be a statistical penalty for combining the results of Part 1 and Part 2 since the results of Part 1 must be analyzed to determine Part 2 dose (alpha spending)?

Answer: We acknowledge that the type I error rate has to be controlled when combining results of Part 1 and Part 2. Therefore, data will be analyzed using the inverse normal method as already described. We now added the reference of Bauer and Köhne describing this method to the section ‘Statistical analyses’ page 16.

• “Where appropriate, predefined sensitivity analysis and exploratory analysis will be employed.” If these analyses are predefined, please define them.

Answer: This statement is related to the analyses of all other secondary endpoint, which are fore exploratory purposes only. We deleted the sentence “Where appropriate...be employed” and describe the analyses of the secondary endpoint in more detail.

• Ethics section should be changed to past tense since IRB has already approved study at the time of this report (“….were approved by the IRB..)

Answer: This is now adjusted as requested by the reviewer.

• More specifics on the role of the sponsor should be added, with relation to interpretation of analyses and drafting of the manuscript.

Answer: This is now described in more detail in the section ‘Potential conflicts of interest’ on page 19.

VERSION 2 – REVIEW

REVIEWER	Frederic T. (Josh) Billings, IV Vanderbilt University Medical Center United States
REVIEW RETURNED	11-Jul-2016

GENERAL COMMENTS	Authors have done a good job improving their manuscript based on initial reviews.