PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	A study protocol for a prospective observational study on the pharmacokinetic properties of the Irrua ribavirin regimen used in routine clinical practice in Lassa fever patients in Nigeria
AUTHORS	Erameh, Cyril; Edeawe, Osahogie; Akhideno, Peter; Eifediyi, Gloria; Omansen, Till; Wagner, Christine; Sarpong, Francisca; Koch, Till; Wicha, Sebastian; Kurth, Florian; Duraffour, Sophie; Oestereich, Lisa; Pahlmann, Meike; Okogbenin, Sylvanus; Ogbaini-Emovon, Ephraim; Günther, Stephan; Ramharter, Michael; Groger, Mirjam

VERSION 1 – REVIEW

REVIEWER	Farhad Kamali Newcastle University, United Kingdom
REVIEW RETURNED	27-Jan-2020

GENERAL COMMENTS	This paper outlines the protocol for a prospective observational trial investigating the PK/PD of a new intravenous dosing regimen for ribavirin (Irrua Ribavirin Regimen) in adult patients with PCR confirmed Lassa fever. The trial is to be conducted at a single centre (ISTH) in Nigeria. The purpose of the study is to provide supportive information on the efficacy of the new dosing regimen in treating patients with Lassa fever. The paper is well written and it is relatively easy to follow the protocol. However, there are several issues that require the authors' attention as follows: The authors state that "from clinical experience the Irrua regimen is postulated to be efficacious yet an easier to use and a safer alternative to the McCormick regimen" (page 12; lines 36-40). What supportive data are there to back this statement? Please provide the information. The primary objective for this study is to establish ribavirin PK for the Irrua regimen. The information is said will help to establish whether ribavirin plasma concentrations reach levels that would be sufficient to exert antiviral effect. Is there information on the range of plasma ribavirin concentration that produces effective antiviral activity? If so then please provide this information. Please use the correct PK terminologies throughout (i.e. maximum plasma drug concentration (Cmax), Time to maximum plasma drug concentration (Tmax), area under the plasma drug concentration versus time (AUC), half-life (t1/2), volume of distribution (Vd). Loading dose of ribavirin- It is stated that if the start dose is > 6g then 2/3 of the dose will be given straight away and the remaining 1/3 is administered 8 hours later (page 12; line 52). On what basis has this division of dosing been selected? What happens if the

	loading dose is <6g? Is the whole dose given as a single injection? If so this needs to be made clear. It is mentioned that data derived from this observational study will serve as basis for further clinical studies studying the efficacy and safety of ribavirin and provide the possibility to compare the Irrua regimen with alternative treatment candidates and regimen (page 12; lines 58-60). For the benefit of the reader please provide some information about these possible alternatives. Given the multitude of side-effects associated with ribarivin there appears to be no mention (both in the study flow chart and the text) of how the adverse effects associated with the treatment will be recorded. Also there is no mention in the flow chart about the multitude of tests carried out under 'secondary objectives' as outlined on page 13; line 43-56. Under 'participant safety' (page 20) the authors suggest that risk to trial participants is minimal and will be mainly associated with repeated blood withdrawals and local pain caused by venepuncture. How about the risks (possible toxic side-effects) associated with the treatment itself which I have previously alluded to? Trial management and safety monitoring:- There is no mention of whether there is a study steering committee in place to monitor study progress and when necessary to take action if recruitment is not within target. It is mentioned in the protocol that "an independent medical monitor will monitor the participants' safety data." (page 20; lines 21-22). I am not convinced this measure is sufficient. It is more appropriate to have a data and safety monitoring board made up of an independent panel of people who can assess any safety issues that need to be brought to the attention of the investigators and the participants. The research group appears to be made up of people from Nigeria and Germany and the study itself is supported by funds from Germany. It would be good to provide some information about the relationship between the Nigerian and German partners in the conduct of this study. Statistical analysis- More detailed information is needed about the statistical test which will be used to evaluate the PK and PD data. Blood sampling and analysis: What exactly is meant by 'leftover material'? (page 18; line 39). Please explain. Regarding viral inactivation prior to sample analysis please provide details for the procedure or cite the appropriate reference paper. It is said that aliquots of samples will be shipped to BNITM "according to UN2814 regulations". Please provide the appropriate reference for this. Population PK model:- The authors state "In particular, we will analyse the changes in ribavirin concentration and hemoglobin levels, alanine aminotransferase (ALT/GPT), and uric acid." (page 23; line 37-39). For the benefit of the reader please provide the reasons for specifically choosing these parameters.

REVIEWER	Dr Rephaim Mpofu University of Cape Town, South Africa
REVIEW RETURNED	28-Jan-2020

GENERAL COMMENTS	This is a review of a research protocol titled "A study protocol for a prospective observational study on the pharmacokinetic properties of the Irrua ribavirin regimen used in routine clinical practice in Lassa fever patients in Nigeria". This research study aims to assess the pharmacokinetic profile of ribavirin treatment in patients diagnosed with Lassa Fever when administered using a modified

	Irrua ribavirin regimen in a referring hospital in Nigeria. This research protocol will provide important data concerning ribavirin therapy and may allow for the improvement of the current regimen which is currently provided 6 hourly for the first 4 days. Specific comments are noted below: Major: 1. Study flow chart: will pregnancy testing be performed for women with childbearing potential prior to ribavirin administration? 2. Page 16, line 34: How will the study team ensure that women do not accidentally fall pregnant in the upcoming 3 months? An important point to note here is that the FDA package insert (for Copegus, used in the treatment of chronic Hepatitis C) recommends that women not fall pregnant for 6 months following ribavirin treatment. 3. Please clarify why is a control arm was not included in the study design? A control arm of patients that receive the standard WHO regimen would allow you to robustly compare the Irrua regimen against the "standard of care" 4. Page 17, limitations: The lack of a standard of care arm is an additional limitation. As your literature review states, the data that the current WHO regimen is based on had issues that may have questioned its validity. This could, therefore, be a chance to assess the standard of care in an African population in the disease of interest (Lassa fever), by which the Irrua regimen may be compared with. If found to be comparable, this regimen may even be favoured due to its improved dosage regimen and other advantages that have been stated. 5. page 28, line 23-31: The lack of financial compensation for participation in this clinical trial is slightly concerning from an ethical perspective. While the hesitation due to the risk of manipulation and coercion is understandable, I believe that this risk needs to be balanced against the number of procedures that a participant may be expected to undergo, as well as the risk that such additional procedures may bring. Even if compensation would not have been provided by financial means, the hesitancy to provide even adjunctive incentives such as phone credits suggests that these items would only be provided in the rarest of circumstances. Inclusion of the price of an impregnated mosquito net may appease the reader and reduce their fears of participant exploitation. 6. Study flow chart: An abbreviated physical examination should be conducted prior to administering the dose, otherwise one cannot exclude a worsening of the patient's clinical condition between screening and administration of the first dose that may affect their eligibility. 7. Will any other indicators of clinical condition be performed as a means of monitoring, e.g. blood pressure? Minor: 8. Page 6, line 15-17: Under weaknesses, it is stated that only patients able to give written informed consent can participate. However, this would exclude illiterate participants for whom you have made alternative provisions through an impartial witness. Consider rephrasing this statement for clarity 9. Page 10, Line 24-27: Consider inserting a Reference. 10. Page 11, Line 4: Insert BNITM expansion (abbreviation explanation)

	11. Literature review: What is known about the PK of ribavirin? Consider referencing at least 2 papers, e.g. Lertora J (1991); Preston S (1999) 12. Page 12, Line 16: Please describe the WHO regimen recommendation for ribavirin treatment 13. Page 12, line 17: What process/rationale was followed in the formulation of the Irrua ribavirin regimen? Were PK parameters calculated and an alternate dosage regimen was formulated based on that? 14. Page 12: Does this mean that a patient diagnosed on day 0 would have to wait 24 hours before they can receive medical intervention? or will adjunctive treatment be administered in the meantime? What is the likelihood of patient decompensation between screening and initiation of ribavirin therapy? 15. Page 13: Tmax = Time to peak/maximum concentration. Consider amending expansion. 16. Page 13, Line 40: AUC = Area under the concentration-time curve. Consider amending expansion 17. Page 13, line 40: T1/2 = Half-life or half-time. Consider amending expansion. 18. Page 14, line 36: Kindly correct reference formatting 19. Page 14, line 48: Expand GCLP at first mention and include in the list of abbreviations 20. Selection criteria: Are patients with Renal failure eligible for inclusion? Severe renal failure may require dose adjustment. Additionally, renal function is a critical factor as it is largely eliminated renally. 21. Page 16, line 13: Please clarify the meaning of the inclusion criterion “anticipated treatment with intravenous ribavirin”. If they are confirmed to have LF, wouldn’t IV ribavirin treatment always be anticipated? 22. Page 16, Exclusion criteria: Consider including Concomitant administration of didanosine and other contraindicated concomitant medication as an exclusion criterion 23. Page 16, line 44-50: This section doesn’t really add anything as this has been explained in the inclusion and exclusion criteria

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1
Reviewer Name: Farhad Kamali
Institution and Country: Newcastle University, United Kingdom Please state any competing interests or state ‘None declared’: None declared

This paper outlines the protocol for a prospective observational trial investigating the PK/PD of a new intravenous dosing regimen for ribavirin (Irrua Ribavirin Regimen) in adult patients with PCR confirmed Lassa fever. The trial is to be conducted at a single centre (ISTH) in Nigeria. The purpose of the study is to provide supportive information on the efficacy of the new dosing regimen in treating patients with Lassa fever.
The paper is well written and it is relatively easy to follow the protocol. However, there are several issues that require the authors’ attention as follows:

The authors state that “from clinical experience the Irrua regimen is postulated to be efficacious yet an easier to use and a safer alternative to the McCormick regimen” (page 12; lines 36-40). What supportive data are there to back this statement? Please provide the information.

Answer: We are grateful to the reviewer for this comment. The Irrua regimen was developed based on empirical considerations and to respond to logistical limitations at the lassa fever treatment sites. This regimen has never been evaluated from a PK perspective, which is the reason for performing this trial. The statement about being efficacious is based on the cohorts of patients that have been treated over the last 10 years at Irrua Specialist Treatment Hospital (ISTH), the largest Lassa fever treatment centre in the world. The regimen has consequently also been endorsed by the Nigerian centre for disease control (NCDC) as recommended regimen for Nigeria. The regimen is easier and safer to use, as the number of doses per day is lower than for the McCormick regimen. Therefore, the exposure of the personnel to LF diseases patients is reduced.

Changes: The section (p12 lines 36-40) has been rephrased according to the suggestions of the reviewer.

The primary objective for this study is to establish ribavirin PK for the Irrua regimen. The information is said will help to establish whether ribavirin plasma concentrations reach levels that would be sufficient to exert antiviral effect. Is there information on the range of plasma ribavirin concentration that produces effective antiviral activity? If so then please provide this information.

Answer: The only information available is data from cell culture and NHP, as stated in the introduction (p 11 lines 13-19). This is one of the reasons for performing this trial.

Changes: No changes

Please use the correct PK terminologies throughout (i.e. maximum plasma drug concentration (Cmax), Time to maximum plasma drug concentration (Tmax), area under the plasma drug concentration versus time (AUC), half-life (t1/2), volume of distribution (Vd).

Answer: We are grateful for these corrections

Changes: terminologies have been changed.

Loading dose of ribavirin- It is stated that if the start dose is > 6g then 2/3 of the dose will be given straight away and the remaining 1/3 is administered 8 hours later (page 12; line 52). On what basis has this division of dosing been selected? What happens if the loading dose is <6g? Is the whole dose given as a single injection? If so this needs to be made clear.

Answer: The division of dosing is based on clinical experience and on empiric/clinical observation of tolerability of ribavirin. There is no published data or official recommendation for it. Indeed, the whole dose is given as single injection if the dose is < 6g.

Changes: The section (page 12; line 52) has been rephrased according to the suggestion of the reviewer.

It is mentioned that data derived from this observational study will serve as basis for further clinical studies studying the efficacy and safety of ribavirin and provide the possibility to compare the Irrua regimen with alternative treatment candidates and regimen (page 12; lines 58-60). For the benefit of the reader please provide some information about these possible alternatives.

Answer: The main potential alternative is currently favipiravir alone or in combination with ribavirin.

Changes: Information about favipiravir as alternative or combination partner for ribavirin has been added (page 12; lines 58-60).

Given the multitude of side-effects associated with ribarivin there appears to be no mention (both in the study flow chart and the text) of how the adverse effects associated with the treatment will be recorded. Also there is no mention in the flow chart about the multitude of tests carried out under 'secondary objectives' as outlined on page 13; line 43-56.
AND
Under 'participant safety' (page 20) the authors suggest that risk to trial participants is minimal and will be mainly associated with repeated blood withdrawals and local pain caused by venepuncture. How about the risks (possible toxic side-effects) associated with the treatment itself which I have previously alluded to?

Answer: This is an observational study as defined in the title, in which the PK characteristics of routine treatment are evaluated. Only description of the concentration profile of ribavirin as used in routine care will take place and no intervention (i.e. decision on dose or treatment) is performed. By definition the potential side effects of a drug not provided from the study is not a study associated risk. The responsible institutional review board therefore asked us to collect only AEs of study related procedures (i.e. phlebotomy, page 20; lines 46-57), of which the treatment is not part.

Changes: No changes.

Trial management and safety monitoring:- There is no mention of whether there is a study steering committee in place to monitor study progress and when necessary to take action if recruitment is not within target. It is mentioned in the protocol that "an independent medical monitor will monitor the participants' safety data." (page 20; lines 21-22). I am not convinced this measure is sufficient. It is more appropriate to have a data and safety monitoring board made up of an independent panel of people who can assess any safety issues that need to be brought to the attention of the investigators and the participants.

Answer: It is very unusual for an observational study to set up a DSMB as per definition no intervention is performed by the study. The Ethics committee recommended toidentify a medical monitor to help guide investigators with regards to inclusion/exclusion criteria. The project management team will of course monitor the progress of the study and will take appropriate action.

Changes: No changes

The research group appears to be made up of people from Nigeria and Germany and the study itself is supported by funds from Germany. It would be good to provide some information about the relationship between the Nigerian and German partners in the conduct of this study.

Answer: There is a long-lasting institutional collaboration between ISTH and BNITM, as stated on page 11 lines 4-7.

Changes: We added come information as suggested by the reviewer in the paragraph on page 11 lines 4-7.

Statistical analysis- More detailed information is needed about the statistical test which will be used to evaluate the PK and PD data.

Answer: Only descriptive evaluation of PK/PD will be performed (page 23 lines 45-53) and no statistical hypothesis testing is envisaged, as this is a secondary outcome .

Changes: No changes

Blood sampling and analysis: What exactly is meant by ‘leftover material’? (page 18; line 39). Please explain.

Answer: we agree that description of “leftover material” should be specified.

Changes: An additional description of what is meant by the term “leftover material” was added on page 18.

Regarding viral inactivation prior to sample analysis please provide details for the procedure or cite the appropriate reference paper.

Answer: a citation of a publication using the employed inactivation method has been added

It is said that aliquots of samples will be shipped to BNITM “according to UN2814 regulations”. Please provide the appropriate reference for this.

Answer/Change: the reference has been added (page 19 line 41).

Population PK model: The authors state “In particular, we will analyse the changes in ribavirin concentration and hemoglobin levels, alanine aminotransferase (ALT/GPT), and uric acid.” (page 23; line 37-39). For the benefit of the reader please provide the reasons for specifically choosing these parameters.

Answer: We agree with the reviewer that giving reasons for choosing these markers will benefit the reader.

Changes: Additional information on the markers were added in the paragraph on page 23 line 37-39.

Reviewer: 2
Reviewer Name: Dr Rephaim Mpofu
Institution and Country: University of Cape Town, South Africa Please state any competing interests or state ‘None declared’: None declared

This is a review of a research protocol titled “A study protocol for a prospective observational study on the pharmacokinetic properties of the Irrua ribavirin regimen used in routine clinical practice in Lassa fever patients in Nigeria”. This research study aims to assess the pharmacokinetic profile of ribavirin treatment in patients diagnosed with Lassa Fever when administered using a modified Irrua ribavirin regimen in a referring hospital in Nigeria. This research protocol will provide important data concerning ribavirin therapy and may allow for the improvement of the current regimen which is currently provided 6 hourly for the first 4 days. Specific comments are noted below:

Major:
1. Study flow chart: will pregnancy testing be performed for women with childbearing potential prior to ribavirin administration?

Answer: We thank the reviewer for this important comment. Pregnancy will be assessed in routine care using history, physical examination, ultrasound examination and if needed pregnancy testing. Of note, also pregnant women receive ribavirin therapy for lassa fever (see PMID 31465848) but would not be included in the study.

Changes: Additional information was added in the section describing exclusion criteria (page 16).

2. Page 16, line 34: How will the study team ensure that women do not accidentally fall pregnant in the upcoming 3 months? An important point to note here is that the FDA package insert (for Copegus, used in the treatment of chronic Hepatitis C) recommends that women not fall pregnant for 6 months following ribavirin treatment.

Answer: As for all women of childbearing potential treated with ribavirin, counselling for appropriate contraceptive measures including condoms, hormonal contraceptives of abstinence will be recommended. As ribavirin treatment is not a study specific procedure, counselling of women will be part of routine care and is not specified explicitly in the protocol.

Changes: No changes.

3. Please clarify why is a control arm was not included in the study design? A control arm of patients that receive the standard WHO regimen would allow you to robustly compare the Irrua regimen against the "standard of care"
AND
4. Page 17, limitations: The lack of a standard of care arm is an additional limitation. As your literature review states, the data that the current WHO regimen is based on had issues that may have questioned its validity. This could, therefore, be a chance to assess the standard of care in an African population in the disease of interest (Lassa fever), by which the Irrua regimen may be compared with. If found to be comparable, this regimen may even be favoured due to its improved dosage regimen and other advantages that have been stated.

Answer: At present we plan to perform an observational study on the Irrua treatment regimen, which is the recommended treatment regimen for patients with Lassa fever in Nigeria as endorsed by the Nigerian centre for disease control (NCDC). We agree with the reviewer that a comparative study will be valuable in the future, but this observational study is in our view a necessary first step to form a basis for future interventional trials.

Changes: A statement discussing the nature and limitations of this observational trial has been added to the Study limitations-section (page 17, lines 17 ff.).

5. page 28, line 23-31: The lack of financial compensation for participation in this clinical trial is slightly concerning from an ethical perspective. While the hesitation due to the risk of manipulation and coercion is understandable, I believe that this risk needs to be balanced against the number of procedures that a participant may be expected to undergo, as well as the risk that such additional procedures may bring. Even if compensation would not have been provided by financial means, the hesitancy to provide even adjunctive incentives such as phone credits suggests that these items would only be provided in the rarest of circumstances. Inclusion of the price of an impregnated mosquito net may appease the reader and reduce their fears of participant exploitation.

Answer: We do understand the concerns of the reviewer and are grateful for the comment. Ethics committee of ISTH advised that financial compensation would constitute an undue enticement to participate in this observational study given the local setting. We trust that the local EC is the best body to judge on this and therefore follow their guidance.

Change: the price of an long-lasting impregnated net was added as suggested by the reviewer (page 28).

6. Study flow chart: An abbreviated physical examination should be conducted prior to administering the dose, otherwise one cannot exclude a worsening of the patient's clinical condition between screening and administration of the first dose that may affect their eligibility.

Answer: We agree with the reviewer and changed the flow chart accordingly.

7. Will any other indicators of clinical condition be performed as a means of monitoring, e.g. blood pressure?

Answer: clinical monitoring (e.g. of blood pressure) will be performed depending on the clinical condition of the patient as part of routine care. As this is not a study specific procedure, the protocol does not explicitly mention it.

Minor:
8. Page 6, line 15-17: Under weaknesses, it is stated that only patients able to give written informed consent can participate. However, this would exclude illiterate participants for whom you have made alternative provisions through an impartial witness. Consider rephrasing this statement for clarity

Answer: We thank the reviewer for this thoughtful comment and changed the manuscript according to his suggestion.

9. Page 10, Line 24-27: Consider inserting a Reference.

Answer: changed according to the suggestion of the reviewer

10. Page 11, Line 4: Insert BNITM expansion (abbreviation explanation)

Answer: Changed according to the suggestion of the reviewer

11. Literature review: What is known about the PK of ribavirin? Consider referencing at least 2 papers, e.g. Lertora J (1991); Preston S (1999)

Answer: Changed according to the suggestion of the reviewer

12. Page 12, Line 16: Please describe the WHO regimen recommendation for ribavirin treatment

Answer: Changed according to the suggestion of the reviewer.

13. Page 12, line 17: What process/rationale was followed in the formulation of the Irrua ribavirin regimen? Were PK parameters calculated and an alternate dosage regimen was formulated based on that?

Answer: The Irrua regimen was developed based on empirical considerations and to respond to logistical limitations at the lassa fever treatment sites. This regimen has never been evaluated from a PK perspective, which is the reason for performing this trial.

14. Page 12: Does this mean that a patient diagnosed on day 0 would have to wait 24 hours before they can receive medical intervention? or will adjunctive treatment be administered in the meantime? What is the likelihood of patient decompensation between screening and initiation of ribavirin therapy?

Answer: Day 0 refers to screening procedure and day 1 refers to start of treatment. Day 0 and day 1 will most likely be the same day, depending on availability of PCR results. The local lab performs PCR twice daily so patients are treated quickly and do not have to wait. This procedure is not influenced by this observational study. All treatment decisions including timing of treatment are taken by the treating physicions as part of standard care.

15. Page 13: Tmax = Time to peak/maximum concentration. Consider amending expansion.
16. Page 13, Line 40: AUC = Area under the concentration-time curve. Consider amending expansion
17. Page 13, line 40: T1/2 = Half-life or half-time. Consider amending expansion.

Joint Answer: all of the above changed according to suggestions of the reviewer.

18. Page 14, line 36: Kindly correct reference formatting

Answer: changed according to the suggestion of the reviewer.

19. Page 14, line 48: Expand GCLP at first mention and include in the list of abbreviations

Answer: changed according to the suggestion of the reviewer.

20. Selection criteria: Are patients with Renal failure eligible for inclusion? Severe renal failure may require dose adjustment. Additionally, renal function is a critical factor as it is largely eliminated renally.

Answer: Dialysis is an exclusion criterion but there are no other exclusion criteria with respect to renal function, therefore a representative sample of patients can be assessed. Decisions on dose adjustments will be taken by the physicians as part of routine care (observational study).
21. Page 16, line 13: Please clarify the meaning of the inclusion criterion “anticipated treatment with intravenous ribavirin”. If they are confirmed to have LF, wouldn’t IV ribavirin treatment always be anticipated?

Answer: there are few exceptions possible, e.g. very mild cases, where oral ribavirin might be used or severe known allergy to ribavirin or refusal of the patient to be treated with ribavirin.

22. Page 16, Exclusion criteria: Consider including Concomitant administration of didanosine and other contraindicated concomitant medication as an exclusion criterion

Answer: changed according to the suggestion of the reviewer.

23. Page 16, line 44-50: This section doesn’t really add anything as this has been explained in the inclusion and exclusion criteria

Answer: This is a short additional explanation of the choice of inclusion criteria.

Changes: No changes

VERSION 2 – REVIEW

REVIEWER	Farhad Kamali Newcastle University, UK.
REVIEW RETURNED	27-Feb-2020

GENERAL COMMENTS	The authors have adequately addressed my concerns/questions.

REVIEWER	Rephaim Mpofu University of Cape Town, South Africa
REVIEW RETURNED	04-Mar-2020

GENERAL COMMENTS	This is a review of a prospective, observational research study that aims to assess the pharmacokinetic profile of a modified ribavirin regimen for the treatment of Lassa fever in a referring hospital in Nigeria. The protocol has been significantly improved, however, there are still a few concerning gaps, particularly around the background knowledge that dictates the scientific rationale for this study, as well as an outline of the data analysis plan: 1. I do not think that the Irrua regimen, as well as the data that informed the formulation of this regimen, has been adequately explained. Someone reading the protocol with no prior knowledge of the history of the Irrua regimen or ribavirin PK would not be able to understand how this regimen came about or be satisfied that this regimen has theoretically similar efficacy when compared with the McCormick regimen. I would hope that ISTH would have considered some data in the formulation of this regimen. I am not sure if clinical experience from the last decade can be deemed sufficient justification for the design of a drug regimen without consideration of what is known at that time. Additionally, the PK profile of ribavirin has still not been described, and there has therefore been no rationalisation of the adequacy of the Irrua regimen. Even healthy volunteer ribavirin PK data would be partially informative, e.g. to know that the half-life of ribavirin is 12 days, and daily dosing would, therefore, be justified. This would also lend credibility to your statement that women of childbearing potential should plan on falling pregnant for the upcoming 3 months to be included in the study. 2. The methodology section still lacks clarity on what statistical methods will be used to analyse the data, especially for the primary objective, i.e. pharmacokinetic profile. Information that is important, but lacking, includes but is not limited to questions such as: Will a non-compartmental analysis be conducted or will a compartmental model be used? What statistical methods will be used to determine the PK parameters? Will medians or means be computed for the PK parameters such as Cmin or Tmax? How will the AUC be determined? Will the participant baseline characteristics be assessed and reported? It would be quite difficult to replicate your proposed data analysis by following the currently described methodology. 3. Please describe how missing data be handled. 4. “Clinical, haematological and biochemical safety and tolerability of the routine Irrua 5. Regimen”. This is an objective, not an endpoint. This statement does not state how these aims/objectives will be

	assessed, e.g. by use of adverse event monitoring, or full blood count to look for haemolysis. 6. Please describe whether adverse events besides those associated with phlebotomy will be assessed and reported as this is not included as an endpoint, nor is it included in the schedule of enrolment & assessments for participants table. If safety is one of your objectives, then I would think that adverse events need to be looked for and reported. One might find, for example, that the incidence of haemolysis is higher (or lower) when the Irrua regimen is used compared to the original regimen. 7. As ribavirin undergoes renal elimination, it is important to consider the patient's renal function when assessing eligibility for a pharmacokinetic, drug disposition, study. If the study team plans on including patients regardless of their renal function, then adequate steps should be taken to account for that and adjust for that in the PK analysis. Otherwise, this could introduce bias in your PK analysis. E.g. 4 patients have deranged glomerular filtration rate, resulting in a decreased elimination by 50% (half-life, therefore, increases by approximately 35%). This would have a significant impact on your calculated half-life. Additionally, if the dose is adjusted, can it really be said that they are still receiving a standard Irrua regimen? Does the irrua regimen provide recommendations for renal adjustment? If so, this needs to be included in the protocol. Minor comments are noted below: • Please review the protocol for typographical errors, particularly the revised sections, e.g. Page 37 of 57, "for 4 dayse followed by 7.5 mg/kg every 8 hours for 6 days". • Will this research study be registered on any other registries, e.g. Pan African clinical trials registry or the Nigeria clinical trials registry? If so, please include them in the protocol.

VERSION 2 – AUTHOR RESPONSE

Reviewer: 1

Reviewer Name: Farhad Kamali

Institution and Country: Newcastle University, UK.

Please state any competing interests or state 'None declared': None declared

Please leave your comments for the authors below

The authors have adequately addressed my concerns/questions.

Reviewer: 2

Reviewer Name: Rephaim Mpofu

Institution and Country: University of Cape Town, South Africa

Please state any competing interests or state 'None declared': None to declare

Please leave your comments for the authors below

This is a review of a prospective, observational research study that aims to assess the pharmacokinetic profile of a modified ribavirin regimen for the treatment of Lassa fever in a referring hospital in Nigeria.

The protocol has been significantly improved, however, there are still a few concerning gaps, particularly around the background knowledge that dictates the scientific rationale for this study, as well as an outline of the data analysis plan:

1. I do not think that the Irrua regimen, as well as the data that informed the formulation of this regimen, has been adequately explained. Someone reading the protocol with no prior knowledge of the history of the Irrua regimen or ribavirin PK would not be able to understand how this regimen came about or be satisfied that this regimen has theoretically similar efficacy when compared with the McCormick regimen. I would hope that ISTH would have considered some data in the formulation of this regimen. I am not sure if clinical experience from the last decade can be deemed sufficient justification for the design of a drug regimen without consideration of what is known at that time. Additionally, the PK profile of ribavirin has still not been described, and there has therefore been no rationalisation of the adequacy of the Irrua regimen. Even healthy volunteer ribavirin PK data would be partially informative, e.g. to know that the half-life of ribavirin is 12 days, and daily dosing would, therefore, be justified. This would also lend credibility to your statement that women of childbearing potential should plan on falling pregnant for the upcoming 3 months to be included in the study.

Response: We thank the reviewer for this comment and agree that the rational and background for the Irrua regimen as it stands is not explained in full depth in the protocol. However, we would like to emphasize that this is not a protocol for an interventional study, introducing a new changed drug regimen, but a protocol for pharmacokinetic evaluation of an existing, established ribavirin regimen. We therefore argue that a “justification” for the drug regimen is not a priority of the protocol.

As stated in the protocol, the Irrua regimen has been used by the largest LF treatment centre worldwide (ISTH) for more than ten years and has been endorsed by the Nigerian centre for disease control (NCDC) as recommended regimen for Nigeria. It has indeed been developed mainly based on empirical considerations and to respond to logistical limitations at the lassa fever treatment sites. One main advantage of the altered regimen is the reduced exposure of personnel to LF patients compared to the WHO regimen, as explained in the “rationale for this project” section. Yet, the regimen has never been evaluated from a PK perspective (which is also true for the WHO regimen), which is the reason for performing this trial.

We agree with the reviewer that additional data on ribavirin PK in other indications might be useful for readers and added this information to the literature review section.

Change: additional data on ribavirin PK and an additional reference was added to the literature review section, as suggested by the reiviewer (page 7 of 35 of the protocol)

2. The methodology section still lacks clarity on what statistical methods will be used to analyse the data, especially for the primary objective, i.e. pharmacokinetic profile. Information that is important, but lacking, includes but is not limited to questions such as: Will a non-compartmental analysis be conducted or will a compartmental model be used? What statistical methods will be used to determine the PK parameters? Will medians or means be computed for the PK parameters such as Cmin or Tmax? How will the AUC be determined? Will the participant baseline characteristics be assessed and reported?

It would be quite difficult to replicate your proposed data analysis by following the currently described methodology.

Reponse: We are grateful for this comment. As usual in clinical studies, details on statistical analyses will be specified in a statistical analysis plan, separate from the protocol.

Concerning the specific questions of the reviewers above:

A non-compartmental analysis will be performed, as specified in the section “description of classical PK parameters”, page 21 of 35.

Participants baseline characteristics will be assessed and reported as stated in the “study procedures” section, subsection “data acquisition”, Page 13 of 35 in the protocol.

Whether medians or means will be used will depend on the distribution of the data, details will be specified in the statistical analysis plan.

AUC will be calculated using the linear trapezoidal method, this information has been added to the section “description of classical PK parameters”, page 21 of 35.

Changes: According to the suggestions of the reviewer, information on the statistical analysis plan and on the calculation of AUC have been added to the protocol as described above.

3. Please describe how missing data be handled.

Response: Missing data will be handled as N.D., the number of available data points will be indicated for each outcome during reporting. Details will be specified in the statistical analysis plan.

Changes: No changes

4. “Clinical, haematological and biochemical safety and tolerability of the routine Irrua

5. Regimen”. This is an objective, not an endpoint. This statement does not state how these aims/objectives will be assessed, e.g. by use of adverse event monitoring, or full blood count to look for haemolysis.

AND

6. Please describe whether adverse events besides those associated with phlebotomy will be assessed and reported as this is not included as an endpoint, nor is it included in the schedule of enrolment & assessments for participants table. If safety is one of your objectives, then I would think that adverse events need to be looked for and reported. One might find, for example, that the incidence of haemolysis is higher (or lower) when the Irrua regimen is used compared to the original regimen.

Response: We are grateful for this comment, which addresses an important aspect of the study. This is an observational study, in which the PK characteristics of routine treatment are evaluated. Only description of the PK profile of ribavirin as used in routine care will take place and no intervention (i.e. decision on dose or treatment) is performed. By definition the potential side effects of a drug not provided from the study is not a study associated risk. The responsible institutional review board therefore asked us to collect only AEs of study related procedures (i.e. phlebotomy, page 20; lines 46-57), of which the treatment is not part. As stated correctly, the safety and tolerability of the Irrua regimen is therefore not an objective.

Change: Safety and tolerability of the Irrua regimen was removed from the endpoint-section of the protocol.

7. As ribavirin undergoes renal elimination, it is important to consider the patient's renal function when assessing eligibility for a pharmacokinetic, drug disposition, study. If the study team plans on including patients regardless of their renal function, then adequate steps should be taken to account for that and adjust for that in the PK analysis. Otherwise, this could introduce bias in your PK analysis. E.g. 4 patients have deranged glomerular filtration rate, resulting in a decreased elimination by 50% (half-life, therefore, increases by approximately 35%). This would have a significant impact on your calculated half-life. Additionally, if the dose is adjusted, can it really be said that they are still receiving a standard Irrua regimen? Does the irrua regimen provide recommendations for renal adjustment? If so, this needs to be included in the protocol.

Response: As stated in the previous response to the reviewer on this topic during revision 1, dialysis is an exclusion criterion but there are no other exclusion criteria with respect to renal function. The reason for this is the aim to study a representative sample of patients with Lassa fever. Decisions on dose adjustments will be taken by the physicians as part of routine care and there are no standard recommendations on dose adjustment according to renal function.

Of course, dose reductions, if any, will be documented and renal function / glomerular filtration rate will be taken into account for PK analysis.

Changes: According to the suggestion of the reviewer, we indicated that we will account for impairment of renal function in pk analysis (page 21 of 35 of the protocol).

Minor comments are noted below:

• Please review the protocol for typographical errors, particularly the revised sections, e.g. Page 37 of 57, "for 4 dayse followed by 7.5 mg/kg every 8 hours for 6 days".

Response: changed according to the suggestion of the reviewer.

• Will this research study be registered on any other registries, e.g. Pan African clinical trials registry or the Nigeria clinical trials registry? If so, please include them in the protocol.

Response: The study was registered at ISRCTN registry, the information has been amended in the protocol.

VERSION 3 – REVIEW

REVIEWER	Rephaim Mpofu University of Cape Town, South Africa
REVIEW RETURNED	18-Mar-2020

GENERAL COMMENTS	The revisions have been noted and are adequate. I have no further comments. Good luck with the rest of the submission.