PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Epirubicin for the Treatment of Sepsis & Septic Shock (EPOS-1): study protocol for a randomized, placebo-controlled Phase IIa dose escalation trial
AUTHORS	Thomas-Ruddel, Daniel; Bauer, Michael; Helbig, Christiane; Schlattmann, Peter; Moita, Luís Ferreira; Ehler, Johannes; Rahmel, Tim; Meybohm, Patrick; Gründling, Matthias; Schenk, Heiko; Köcher, Thomas; Brunkhorst, Frank; Heger, Ann-Julika; Weis, Sebastian; Gräler, Markus; study group, EPOS-1; TrialsGroup, SepNetCriticalCare

VERSION 1 – REVIEW

REVIEWER	Black, Lauren Page University of Florida Shands Jacksonville Medical Center, Emergency Medicine
REVIEW RETURNED	12-Jan-2024

GENERAL COMMENTS	General/Major There is a fair amount of verb-tense disagreement, dangling modifiers, etc. Recommend a grammatical edit for English grammar. It does not influence or limit the ability to understand the scientific premise of the manuscript, but is slightly distracting. The manuscript is clearly understandable, but this should be improved prior to publication. Two examples are noted below: - i.e. (Both from abstract): While targeting sepsis-causing pathogens with source control or antimicrobials HAVE had a dramatic impact on morbidity and mortality of sepsis patients, it remains insufficient for about one-third of the affected individuals that still succumb (incomplete sentence). - i.e. the anthracycline epirubicin promoted tissue damage control and lessenED (not lessen) severity of sepsis The authors should be explicit about diagnostic criteria used for sepsis/septic shock. (i.e. SOFA score greater than or equal to 2). This should be in the box and the main text. The authors should consider adding more support for their sample size, more robust sample size calculations, and stronger rationale for the doses chosen. Abstract Would recommend putting the target sample size in the abstract Introduction

	Anthracyclines, a class of drugs used in chemotherapy for over 30 years, have been shown to enhance disease tolerance when given in low doses. - This has no source and is a pretty bold statement that should have a source referenced. - Is there evidence of decreased rates of sepsis/infection among cancer patients who receive anthracyclines? "…has been used at doses up to 30 mg/m2 without toxicity in earlier studies with cancer patients." - Needs a source. "…is a higher dosage then what is intended in the EPOS-1 trial." - Be explicit about the rationale for the specific doses used and what determines/ what is the criterion for "low" dose. Methods The authors propose epirubicin or placebo in a 4:1 ratio. Please be clear about the rationale and justification for the 4:1 ratio. Consider reporting even the single episodes of myelosuppression (i.e. those that do not meet the criteria of 2 or more consecutive days). "we will assign eight patients to epirubicin and two to placebo. Based on the assumption that the probability of a myelotoxicity is 18% the probability of observing at least one myelotoxicity out of eight verum-treated patients equals 79.6% based on a binomial distribution. Thus, a total of 30 (8 x verum vs. 2 x placebo from each phase)patients that reach the 14-day safety endpoint is required…" - What is the assumption for the 18% probability of myelotoxicity based upon? Is there any data that supports this? - Would consider reporting traditional alpha and beta for the sample size calculation. - Concern this may be under-powered. Limitations/Strengths - Would consider including more limitations than just not being powered to assess mortality. Discussion "along with focus sanitation", I think the authors mean "source control"

VERSION 1 – AUTHOR RESPONSE

Reviewer:

Reply from the authors: We thank Dr. Black for the assessment of our manuscript. We have changed the raised points accordingly. We would like to reply as follows:

2.1 There is a fair amount of verb-tense disagreement, dangling modifiers, etc. Recommend a grammatical edit for English grammar. It does not influence or limit the ability to understand the

scientific premise of the manuscript, but is slightly distracting. The manuscript is clearly understandable, but this should be improved prior to publication. Two examples are noted below:

- i.e. (Both from abstract): While targeting sepsis-causing pathogens with source control or antimicrobials HAVE had a dramatic impact on morbidity and mortality of sepsis patients, it remains insufficient for about one-third of the affected individuals that still succumb (incomplete sentence).
- i.e. the anthracycline epirubicin promoted tissue damage control and lessenED (not lessen) severity of sepsis

Reply from the authors: We have carefully revised the language of our manuscript and correct the grammar at several places.

2.2 The authors should be explicit about diagnostic criteria used for sepsis/septic shock. (i.e. SOFA score greater than or equal to 2). This should be in the box and the main text.

Reply from the authors: We added this information in Box 1 and in the main text. It reads now in the Methods section: "**Sepsis is defined following the Sepsis-3 criteria as infection-associated organ dysfunction, represented by an increase in the Sequential Organ Failure Assessment (SOFA) score of two points or more**".

2.3 The authors should consider adding more support for their sample size, more robust sample size calculations, and stronger rationale for the doses chosen.

Reply from the authors: This section has been extended.

2.4 Abstract
Would recommend putting the target sample size in the abstract.

Reply from the authors: We have added the sample size to the abstract as requested.

2.5 Introduction
Anthracyclines, a class of drugs used in chemotherapy for over 30 years, have been shown to enhance disease tolerance when given in low doses.
- This has no source and is a pretty bold statement that should have a source referenced.

Reply from the authors: We apologize for this misunderstanding. The explanation is provided in the section that directly follows. We are now referring to the original manuscript by Dr. Moita and have added references for the statement that anthracyclines are used for over 30 years.

2.6 Is there evidence of decreased rates of sepsis/infection among cancer patients who receive anthracyclines?

Reply from the authors: No, there is no evidence that supports this assumption. The protective effects of epirubicin in the animal model were observed when much lower doses of the drug were given then usually are being used in cancer therapy. We assume that this is caused by hormetic effects while being associated with less side effects of the agent. Our protocol also differs in that we propose a single administration while patients with cancer are treated with multiple cycles of chemotherapy that often include anthracyclines.

2.7 "…has been used at doses up to 30 mg/m^2 without toxicity in earlier studies with cancer patients."
- Needs a source.

Reply from the authors: The appropriate reference has been added.

2.8 "…is a higher dosage then what is intended in the EPOS-1 trial."
- Be explicit about the rationale for the specific doses used and what determines/ what is the criterion for "low" dose.

Reply from the authors: We have altered this section. It reads now: "The epirubicin dosage in Phase 1 is 25% of the dosage applied in the mouse models, *i.e.* 3.75 mg/m^2 that corresponds to approximately 4% of the epirubicin dosage applied in a single course of chemotherapy. If this dose is safe it will be escalated to 7.5 mg/m^2 and finally 15 mg/m^2 which corresponds to approximately 16% of the dosage applied in chemotherapy. It equals the dose that showed benefit in the mouse model and would be the dose to use in a future phase III trial. We expect that none of the applied dosages of epirubicin will increase toxicity in patients with sepsis.We expect that none of the applied dosages of epirubicin will increase toxicity in patients with sepsis."

2.9 Methods
The authors propose epirubicin or placebo in a 4:1 ratio. Please be clear about the rationale and justification for the 4:1 ratio.

Reply from the authors: Thank you for your thoughtful comment. The objective of this trial is to assess safety of low-dose epirubicin as an adjunctive therapy for patients with sepsis and septic shock. Since sepsis can lead to myelosuppression in itself we include a placebo control. Furthermore, since our trial is a first in human trial with regard to epirubicin and sepsis we use a common Phase I clinical design with patients randomized in a 4:1 ratio. such that eight subjects receive the active therapy and two subjects receive placebo. Since we want to see a safety signal more patients are allocated to epirubicin in comparison to placebo.

2.10 Consider reporting even the single episodes of myelosuppression (i.e. those that do not meet the criteria of 2 or more consecutive days).
"we will assign eight patients to epirubicin and two to placebo. Based on the assumption that the probability of a myelotoxicity is 18% the probability of observing at least one myelotoxicity out of eight verum-treated patients equals 79.6% based on a binomial distribution. Thus, a total of 30 (8 x verum vs. 2 x placebo from each phase)patients that reach the 14-day safety endpoint is required…"
- What is the assumption for the 18% probability of myelotoxicity based upon? Is there any data that supports this?
- Would consider reporting traditional alpha and beta for the sample size calculation.
- Concern this may be under-powered.

Reply from the authors: Again, thank you for your remark. Sample size estimation is not based on formal hypothesis testing, where the definition of the acceptable type I and type II error is required. We calculate the binomial probability to observe a least one myelotoxicity out of 8 patients given that the probability for a myelotoxicity equals 18%. The logic is as follows: The binomial probability to observe no myelotoxicity in this setting equals 20.4%. Hence, the probability to observe at least one event equals 100%-20.4% = 79.6%. Thus, in our opinion the study is not underpowered, since we are able to see a signal with considerable probability.

The assumption for the 18% probability of myelotoxicity is based on previous data from cancer patients. It reads now "Since sepsis patients are potentially more susceptible to side effects and altered drug toxicity, we base our sample size calculations on data from cancer patients. These receive four times higher doses of epirubicin (1, 2). Myelotoxicity was observed in cancer patients that received repetitive courses of epirubicin. Herait *et al.* reported grade 3 to 4 leucopenia in approximately 20% of patients that were treated every 3-4 weeks using a dose of

85 to 90 mg/m^2 epirubicin (1). In another trial, myelotoxicity grade 3 to 4 using the WHO classification was reported in 14 % of patients receiving 71-75 mg/m^2 every 3 weeks (2).“

2.11 Limitations/Strengths
- Would consider including more limitations than just not being powered to assess mortality.

Reply from the authors: We have added two more limitations to the table.

2.12 Discussion
“along with focus sanitation”, I think the authors mean “source control”

Reply from the authors: This has been changed.

References

1. Herait P, Poutignat N, Marty M, Bugat R. Early assessment of a new anticancer drug analogue--are the historical comparisons obsolete? The French experience with pirarubicin. Eur J Cancer. 1992;28A(10):1670-6.

2. Ganzina F, Di Pietro N, Magni O. Clinical toxicity of 4'-epi-doxorubicin (epirubicin). Tumori. 1985;71(3):233-40.