PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	PipEracillin Tazobactam versus mERoPENem for treatment of bloodstream infections caused by third generation cephalosporin-resistant Enterobacteriaceae – a study protocol for a non-inferiority open label randomized controlled trial (PeterPen)
AUTHORS	Bitterman, Roni; Koppel, Fidi; Mussini, Cristina; Geffen, Yuval; Chowers, Michal; Rahav, Galia; Nesher, Lior; Ben-Ami, Ronen; Turjeman, Adi; Huberman Samuel, Maayan; Cheng, Matthew; Lee, Todd; Leibovici, Leonard; Yahav, Dafna; Paul, Mical

VERSION 1 – REVIEW

REVIEWER	Nick Daneman Sunnybrook Health Sciences, University of Toronto, Canada I have no competing interests. I have collaborated with multiple investigators involved in this study.
REVIEW RETURNED	22-Jul-2020

GENERAL COMMENTS	PeterPen is a multicentre, open-label, randomized controlled trial designed to test whether piperacillin-tazobactam is non-inferior to meropenem for treatment of bloodstream infections caused by third generation cephalosporin-resistant Enterobacteriaceae. The co-primary outcomes are 30d mortality and 7d treatment failure. The sample size calculations indicate that 542 patients per-arm (total 1084 patients) are required to establish non-inferiority with an absolute margin of -5% for 30d mortality. The trial is clinically important, rigorously designed and conducted by a team with an outstanding track record in the field of bacteremia. Thoughts for consideration: -I agree with the investigators that all RCTs require replication; my personal prediction is that it will reconfirm superiority (or lack of non-inferiority) for piperacillin-tazobactam for these patients and that the clinical community will then need to focus on other carbapenem-sparing strategies such as minimization of unnecessary carbapenem use as empiric therapy in patients with low risk of ESBL, and minimization of antibiotic treatment durations in those with established ESBL infections. But, I look forward to learning the results, and if it establishes non-inferiority it could lead to very significant and important changes in practice. -To change practice, the investigators will need to ensure that the PeterPen design does not favour non-inferiority. In the introduction (pages 5/6) the team outlines some aspects of the MERINO design that favoured non-inferiority - recruitment of patients with

	mild sepsis, the relatively short duration of the intervention, and contamination during the empiric treatment stage. Are these same factors not inherent to PeterPen? -The 72h recruitment window seems appropriate to minimize the empiric pre-randomization contamination with other agent(s), but what is the average time from blood culture collection to confirmation of cephalosporin sensitivity at each site? How much time will be available for screening and inclusion? -The 'use of other antibiotics will not be allowed in the first week of treatment'. How will the team handle situations in which a secondary infection occurs, such as with another Gram negative organism, or with a pip.-tazo./meropenem-resistant Gram positive organism such as MRSA? -Why did the study team choose to make 7d treatment failure a co-primary outcome rather than a secondary outcome? Is that because to consider pip.-tazo. to be non-inferior they require it to be non-inferior to both of these outcomes? -the calculated sample size required for 7d treatment failure is much lower (232pts per arm) than the sample size calculated for the mortality outcome; why not choose a smaller non-inferiority margin for this treatment failure outcome given that PeterPen will be enrolling more than 500 patients per arm? -would it be worthwhile to add carriage of ESBL Enterobacteriaceae as a secondary outcome, in addition to carriage of CRE? -The introduction suggests that early trial termination can lead to spurious results. The protocol states that interim analyses will be conducted at 250, 500 and 750 patients. Will the Peterpen interim rules differ from that of MERINO, or is there a similar risk of termination?

REVIEWER	Adam Stewart University of Queensland, Australia
REVIEW RETURNED	29-Aug-2020

GENERAL COMMENTS	Specific Comments Locations and countries need to be updated on the clinicaltrials.gov trial registry Inclusion criteria – What is the criteria for “evidence of infection”? Exclusion criteria – “Patients with prior bacteraemia” that have not completed therapy – I think this has to be better defined as durations and approaches differ and often these patients have recurrent bloodstream infections Excluding patients who are shocked – why is it that you exclude them at randomisation and not when the index blood culture was taken? In the background and rationale authors identify that they will standardise microbiological methods for this trial, however, in the methods section it states that local methods will be used to enrol patients and that the primary analysis will be in this population. A standardised format will be used at a reference lab. How is this different to MERINO? What website will be used for randomisation? “The study drug will be administered for a minimum of four to five days to complete at least seven days of antibiotic treatment” – This needs to be more strict in its definition. E.g. What constitutes a protocol violation – 4, 5 or 6 days of IV antibiotic? What about the use of antibiotic with only Gram positive activity (e.g. Vancomycin)

	Sample size calculation – I think assuming an overall mortality of 12.5% (~5% more than MERINO) when you are excluding those with shock and those likely to die in 48 h is not justified. A mortality rate of 5% in an RCT setting is more reasonable. 25% treatment failure in the control group – How was this number generated? Seems very high Who will be in the DSMB? Will BMD testing be performed by individuals blinded to trial outcomes? Please explicitly state this SPIRIT Checklist Title – needs to include open-label; needs to be “third generation cephalosporin-resistant”; consider use of Phase 4 Protocol version and date required Roles and responsibilities – Who is the study sponsor? No trial roles and responsibilities documented Outcomes – need to provide more evidence for clinical relevance for chosen endpoints Recruitment – not entirely clear who will be driving this (e.g. the lab or the researchers, how will BSI be identified and flagged?) Data collection methods – need to give more information about how going to complete 90 day follow-up for everyone (this will be difficult) Data monitoring – need to explicitly state composition of DMC Harms – need to state how collecting, assessing, reporting, and managing AEs Dissemination policy – Not mentioned

REVIEWER	Milo Gatti Pharmacology Unit, Department of Medical and Surgical Sciences, Alma Mater Studiorum University of Bologna, Italy
REVIEW RETURNED	30-Aug-2020

GENERAL COMMENTS	The authors aim to replicate the MERINO trial, in order to compare piperacillin-tazobactam with meropenem for the treatment of bloodstream infections caused by ESBL. Given the controversies emerged after publication of MERINO trial and subsequent subanalyses, I think that replication is necessary. The authors explain clearly the rationale for replication in their study protocol. Particuarly, the indiscriminate use of carbapenems in areas where CRE are endemic could be dangerous for public health in terms of increase in carbapenem-resistant isolates. The protocol is very clear in all sections, and I particularly appreciated the paragraph concerning PK/PD considerations, given the extreme importance of a pharmacological optimization of antimicrobials especially in critical care patients affected by MDR infections, due to the several and well-known PK alterations in this setting. In this regard, I have only two comments: * I understand the challenging in individualized dosing when different sites are involved in a multicenter trials. However, a PK/PD assessment could be performed "a posteriori" by the referral center through the collcetion of a single sample in each patients at steady state (given the extended infusion), in order to evaluate whether adequate concentrations of piperacillin-tazobactam or meropenem are achieved with respect to the MIC * Dosing schedule for CRRT sholud be revised according to recent evidence, particularly in patients on CRRT with high effluent flow rate (> 2.5 L/h)

VERSION 1 – AUTHOR RESPONSE

Reviewer 1

1. To change practice, the investigators will need to ensure that the PeterPen design does not favour non-inferiority. In the introduction (pages 5/6) the team outlines some aspects of the MERINO design that favoured non-inferiority - recruitment of patients with mild sepsis, the relatively short duration of the intervention, and contamination during the empiric treatment stage. Are these same factors not inherent to PeterPen?
   Comment: some of these factors are indeed inherent to PETERPEN as well as a pragmatic trial, especially the short treatment duration and contamination during the empiric phase. However, we do not plan to allow contamination in the de-escalation phase. Specifically, patients assigned to PTZ will not cross over to ertapenem for continuation of definitive therapy, as in MERINO; we will make an effort that patients will complete therapy with the assigned regimen. Patients with severe sepsis are not excluded from our study. Also, the consent process in Israel allows to recruit patients using the consent of a physician independent from the study. This process enables recrutiment of severly septic patients. Still, it is hard to predict how many there will eventually be We believe the major strength of PETERPEN is the large sample size and hope this will overcome the above mentioned limitations.
2. The 72h recruitment window seems appropriate to minimize the empiric pre-randomization contamination with other agent(s), but what is the average time from blood culture collection to confirmation of cephalosporin sensitivity at each site? How much time will be available for screening and inclusion?
   Comment: For most sites, time from culture collection to susceptibility results is 24 to 48 hours. This leaves at least 24 hours for enrollment. Although this is a tight schedule we preferred not to enlarge this window to minimize between group contamination.
3. The 'use of other antibiotics will not be allowed in the first week of treatment'. How will the team handle situations in which a secondary infection occurs, such as with another Gram negative organism, or with a pip-tazo/meropenem-resistant Gram positive organism such as MRSA?
   Comment: patients with another Gram negative or a resistant Gram positive known before randomization will be excluded. If such infections arise after randomization and during active treatment with study drugs they will be treated according to physician's discretion. From our experience this is very rare in the first week of Gram-negative bacteremia.
4. Why did the study team choose to make 7d treatment failure a co-primary outcome rather than a secondary outcome? Is that because to consider pip-tazo to be non-inferior they require it to be non-inferior to both of these outcomes?
   Comment: we added treatment failure as a co-primary outcome to allow a stopping point should the mortality be much lower than expected (as occurred in MERINO) and to allow a pre-planned alternative primary outcome for the trial.
5. the calculated sample size required for 7d treatment failure is much lower (232pts per arm) than the sample size calculated for the mortality outcome; why not choose a smaller non-inferiority margin for this treatment failure outcome given that PeterPen will be enrolling more than 500 patients per arm?
   Comment: we believe that a non-inferiority margin of 10% is reasonable for an outcome of treatment failure. Our intention was to reach different sample sizes for failure and mortality, to plan ahead for the case of slow recruitment (bacteria becoming resistant to PTZ) or low mortality rates.
6. Would it be worthwhile to add carriage of ESBL Enterobacteriaceae as a secondary outcome, in addition to carriage of CRE?
   Comment: since the inclusion criteria for the study is a bloodstream infection with ESBL-producing bacteria, the overwhelming majority of patients will be carriers of ESBL Enterobacteriaceae. We do not expect the therapy to eradicate colonization and in the trial sites we do not have capacity to test for carriage of ESBLs without specific funding.

The introduction suggests that early trial termination can lead to spurious results. The protocol states that interim analyses will be conducted at 250, 500 and 750 patients.
Will the Peterpen interim rules differ from that of MERINO, or is there a similar risk of termination?
Comment: The terminations rules are similar, but scheduled later than the MERINO trial. This was a requirement of our ethics committee.

Reviewer 2

1. Locations and countries need to be updated on the clinicaltrials.gov trial registry
   Comment: done.
2. Inclusion criteria – What is the criteria for "evidence of infection"?
   Comment: the criteria are specified in the appendix of the original protocol and are based on the FDA published guidance for industry funded clinical trials. These criteria are specified for UTI, IAI, ABSSSI, CRBSI, CAP, HAP.
3. Exclusion criteria – "Patients with prior bacteraemia" that have not completed therapy – I think this has to be better defined as durations and approaches differ and often these patients have recurrent bloodstream infections
   Comment: we exclude patients with prior infections still necessitating antibiotics since including them will cause contamination of treatment groups with other antibiotics. We include the patients with recurrent bloodstream infections. Although these patients are at risk for more recurrent infections/bacteremia excluding them a priori will decrease the number of eligible patients and will distance the trial from clinical practice.
4. Excluding patients who are shocked – why is it that you exclude them at randomisation and not when the index blood culture was taken?
   Comment: Our assumption is of non-inferiority. All Israeli centers were prepared to recruit also patients with shock both when the index culture was taken and at randomization. Outside Israel, investigators preferred not to include hemodynamically unstable patients. We reached a consensus that if patients were in shock initially and stabilized by the time of randomization, we will include them.
5. In the background and rationale authors identify that they will standardise microbiological methods for this trial, however, in the methods section it states that local methods will be used to enrol patients and that the primary analysis will be in this population. A standardised format will be used at a reference lab. How is this different to MERINO?
   Comment: we will use local labs during trial run and will re-analyse isolates in a central lab using BMD in batch at the end of the trial. We agree that this is no different than the MERINO, however, as this is a pragmatic and currently non-funded trial it will be impossible to apply central laboratory methods (such as BMD) to all local labs participating. We will analyse results both according to local and central lab results. Given the MERINO results we will reach a conclusion only following the BMD susceptibilities will be available.
6. What website will be used for randomisation?
   Comment: we will use RedCap for randomization. Added.
7. "The study drug will be administered for a minimum of four to five days to complete at least seven days of antibiotic treatment" – This needs to be more strict in its definition. E.g. What constitutes a protocol violation – 4, 5 or 6 days of IV antibiotic?
   Comment: treatment duration should be at least 7 days of appropriate antibiotic treatment. E.g. if a patient was treated with 3 days of appropriate empirical treatment he will need at least 4 days of study drug. There is no upper limit, however the lower limit must be maintained.
8. What about the use of antibiotic with only Gram positive activity (e.g. Vancomycin)
   Comment: that is prohibited as per study protocol. We avoided recruitment of patients with polymicrobial infections to reduce contamination of the treatment groups. Assuming this is known prior to randomization these patients should not be enrolled.
9. Sample size calculation – I think assuming an overall mortality of 12.5% (~5% more than MERINO) when you are excluding those with shock and those likely to die in 48 h is not justified. A mortality rate of 5% in an RCT setting is more reasonable.
   Comment: Indeed, this is a concern and for this reason we defined a co-primary outcome of

treatment failure that will occur with a higher frequency. However, as a pragmatic trial with few exclusion criteria, very different from registration trials, we do plan to include a broad spectrum of patients in terms of baseline comorbidities, functional status, and levels of immunosuppression. In AIDA (PMID: 29456043) we recruited patients into a RCT with similar inclusion criteria and 44% mortality at 28 days (albeit with carbapenem-resistant Gram negative bacteria infections)

10. 25% treatment failure in the control group – How was this number generated? Seems very high
    Comment: This was based on our experience in recruiting patients into a trial comparing 7 vs. 14 days of treatment for Gram-negative bacteremia (PMID: 30535100), in which we excluded patients that were febrile or hemodynamically unstable at day 7 (corresponding to the treatment failure definition). The percentage was approximtely 25%
11. Who will be in the DSMB?
    Comment: We invited two ID physicians with experience in leading and performing clinical trials and one pharmacologist also experienced in antibiotic trials. All are external to the study centers and outside of Israel. We added this to the text.
12. Will BMD testing be performed by individuals blinded to trial outcomes? Please explicitly state this
    Comment: BMD will be performed by individuals blinded to trial outcomes and to the local AST results. This has been clarified in the text.
13. SPIRIT Checklist
    a. Title – needs to include open-label; needs to be “third generation cephalosporin-resistant”; consider use of Phase 4
       Comment: done, we did not add phase 4 since this is an investigator-initiated trial that is not part of the regulatory pathway.
    b. Protocol version and date required
       Comment: The protocol version is kept in our files, if necessary we can add to this manuscript.
    c. Roles and responsibilities – Who is the study sponsor? No trial roles and responsibilities documented
       Comment: we added a definition of the sponsor to the manuscript and address roles and responsibilities under the different sections of the manuscript. In more details, we addressed roles and responsibilities in the SPIRIT checklist.
    d. Outcomes – need to provide more evidence for clinical relevance for chosen endpoints
       Comment: We noted in the manuscript that we based the primary outcome selection on proposed primary endpoints for use in clinical trials that compare treatment options for bloodstream infection in adults that were reached through consensus (PMID 27810466). We believe that a discussion on the appropriate outcome measures and their clinical relevance is beyond the scope of the protocol. It is an interesting discussion from the viewpoint of clinicians and patients
    e. Recruitment – not entirely clear who will be driving this (e.g. the lab or the researchers, how will BSI be identified and flagged?)
       In all trial centers the lab reports the identification of Gram-negative bacteria in real time and the research assistants at each site apply inclusion/ exclusion criteria on all Gram-negative BSI reports. We added the identification strategy
    f. Data collection methods – need to give more information about how going to complete 90 day follow-up for everyone (this will be difficult)
       Comment: We clarified that the 90-day follow-up will not require study visits post discharge, but only in-hospital follow-up during the index hospitalization and re-admissions, together with the survival monitoring through national and regional databases. Our outcomes do not require post-discharge visits.
    g. Data monitoring – need to explicitly state composition of DMC
       Comment: Added in text the composition, as above.

h. Harms – need to state how collecting, assessing, reporting, and managing AEs
Comment: added under assessment and follow-up. We will not intervene and leave the decision whether to continue the assigned antibiotic to the treating physicians.

Dissemination policy – Not mentioned
Comment: added in text that in any case results will be made publicly available on the trial registry site. We will of course attempt publication and presentation in international conferences.

Reviewer 3

1. I understand the challenging in individualized dosing when different sites are involved in a multicenter trials. However, a PK/PD assessment could be performed "a posteriori" by the referral center through the collcetion of a single sample in each patients at steady state (given the extended infusion), in order to evaluate whether adequate concentrations of piperacillin-tazobactam or meropenem are achieved with respect to the MIC
Comment: Unfortunately we do not have the infrastructure to perform drug level testing in the different study centers (for now a single center performs meropenem levels) and we do not have the funding to transport samples to a central lab in real time. However, it is a good idea to collect the samples and keep them frozen for a later analysis. We added a sampling time point after the 3rd dose.
2. Dosing schedule for CRRT should be revised according to recent evidence, particularly in patients on CRRT with high effluent flow rate (> 2.5 L/h)
Comment: there are indeed new data coming up continuously on drug dosing in CRRT, also with changing CRRT modalities. We decided to base the dose on a recent literature review of the literature providing recommendations for dosing by effluent flow rate: https://doi.org/10.3389/fphar.2020.00786. We refer in the protocol to this publication and will prescribe dosing in the trial according to the table provided in these recommendations.

VERSION 2 – REVIEW

REVIEWER	Nick Daneman Sunnybrook Health Sciences Centre, University of Toronto
REVIEW RETURNED	29-Oct-2020

GENERAL COMMENTS	I appreciate the authors' response to my questions and comments, and I look forward to learning the results of the trial.

REVIEWER	Adam Stewart University of Queensland Centre for Clinical Research Australia
REVIEW RETURNED	31-Oct-2020

GENERAL COMMENTS	No additional comments

REVIEWER	Milo Gatti University of Bologna, Italy
REVIEW RETURNED	31-Oct-2020

GENERAL COMMENTS	The authors considered the revision suggestions, providing adequate justifications and clarification to all the points raised. I think that the manuscript is appropriate for publication.