PEER REVIEW HISTORY

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ARTICLE DETAILS

TITLE (PROVISIONAL)	Multicenter open-label randomized controlled trial to compare colistin alone with colistin plus meropenem for the treatment of severe infections caused by carbapenem-resistant Gram-negative infections (AIDA): study protocol
AUTHORS	Dickstein, Yaakov; Leibovici, Leonard; Yahav, Dafna; Eliakim-Raz, Noa; Daikos, George; Skiada, Anna; Antoniadou, Anastasia; Carmeli, Yehuda; Nutman, Amir; Levi, Inbar; Adler, Amos; Durante-Mangoni, Emanuele; Andini, Roberto; Cavezza, Giusi; Mouton, Johan; Wijma, Rixt; Theuretzbacher, Ursula; Friberg, Lena; Kristoffersson, Anders; Zusman, Oren; Koppel, Fidi; Dishon, Yael; Altunin, Sergey; Paul, Mical

VERSION 1 - REVIEW

REVIEWER	Rupali Jain University of Washington, Seattle, WA USA
REVIEW RETURNED	01-Dec-2015

GENERAL COMMENTS	Overall, a sound study design. I am concerned that it will be difficult to show superiority with this high mortality disease but if you are able to recruit the number of pts that you anticipate, then it may be okay. A couple items to consider: I would recommend a minimum duration of therapy of 14 days for these serious infections. Clarify infusion time of meropenem dosing for Crcl < 50. Clarify the intent of obtaining colistin and meropenem PK samples. I would recommend additional colistin PK sampling. (current reference is 30, but I think you meant 31) The garzonik study collected samples on day 3 or 4, based on the long half-life. Consider obtaining levels on day 3 or 4.

REVIEWER	Evelyn Shaw Perujo Hospital Universitari de Bellvitge-IDIBELL. Hospitalet de Llobregat, Barcelona, Spain
REVIEW RETURNED	17-Jan-2016

GENERAL COMMENTS	This study deals with a very important issue because of the paucity of good studies with clinical data on the use of combination antimicrobial therapy in the treatment of Carbapenem-resistant Gram negative infections, an increasing threat worldwide. Results from this study may provide important answers to the scientific community.

	1.-Key words: the study is focused on carbapenem-resistant Gram negative infections and Key words section doesn't include this word. They used multidrug resistant gram negative bacteria which is not exactly the same. Maybe will be better change the word to carbapenem-resistant? 2.-Abstract: primary outcome says is a composite variable assessed at day 14: survival + hemodynamic stability + clinical stability **"or"** improvement. But in outcomes section the primary outcome is defined as patient alive at day 14 + hemodynamic stability + **"and"** improvement of SOFA. I think given the strong interest of the study that the definition of primary outcome has to be strictly defined, it should not allow different interpretation of data. Again, as I said in the inclusion/exclusion criteria section, to list the outcomes followed of a definition section could help a better understanding of this section. Currently is a little bit confused. 3.-Objectives and Trial design: the authors wrote, the primary objective is to show superiority..... in the treatment of patients infected with MDR GNB. It will be better change MDR GNB per Carbapenem- resistant GNB which is the aim of the study. 4.-Objectives and Trial design: objectives should be defined more accurately. How researchers have defined the concept of "superiority" in the study? How is planned to be measured, Any specific outcome?. They are interested in any other clinical or microbiological objectives related to the concept of "superiority". The authors also wrote just one secondary objective related to pharmacokinetic models of colistin. This means they are going to measure Colistin steady-state plasma concentrations To define "the specific objectives" is recommended for a better understanding of the study 5.-The inclusion/exclusion criteria will be easier to understand if they be listed. Currently the section of inclusion criteria includes exclusion criteria!. Definitions should be written in a section apart (as it is for example table 1). Does the study include within the inclusion criteria the signed informed consent? And pregnancy test in fertile women? 6.-Table 1 includes UTI definition which is not the current definition of cUTI, one of the populations in this study. Which disease is wanted to be included, UTI or cUTI?. 7.-To get an easier understanding it would be better to separate in the intervention section: arm 1 colistin /arm 2 Colistin + meropenem. Dot apart, put the duration of therapy and antibiotics permitted and not permitted. 8.- The study contemplate criteria for discontinuing or modifying

	allocated interventions for a given trial participant? 9.- Outcome section: in Abstract section, primary outcome says “is a composite variable assessed at day 14: survival + hemodynamic stability + clinical stability **“or”** improvement”. But in outcomes section the primary outcome is defined as patient alive at day 14 + hemodynamic stability + **“and”** improvement of SOFA. I think, given the high interest of this study, the definition of primary outcome has to be strictly defined, it should not allow different interpretation of the data. Again, as I said in the inclusion/exclusion criteria section, listing the outcomes followed by a definition section could help a better understanding of this section. Currently the section is written in a very confused way. It will be also appreciated specify in simpler way, what is considered success and what is failure in the study. 10.- Timeline for follow up: I understand all patients will be followed during 28 days, it does not depend on total duration of therapy?. I understand table 4 includes visits for participants? Could it be better specified? Maybe as an appendice and just include a summary in the text. 11.- How is expected to recruit the patients? How they will be identified in each hospital? 12.- Data collection and microbiological sampling: I’m concerned because authors put in the document “other samples are obtained as clinically indicated”. What does this means?. A clinical trial should have very well defined variables to allow all the investigators to do things in the same way. 13.- Statistical analysis: significance is considered only if p= 0.05 or ≤ 0.05? 14.- Safety monitoring: Is there any adverse event of particular interest in the study?. If yes, specify please. 15.- Discussion: There is a huge discussion related to “Ethics” and I’m not sure if it’s needed in this section. However, I think the final decision is on the Editor

VERSION 1 – AUTHOR RESPONSE

Rupali Jain

1. I would recommend a minimum duration of therapy of 14 days for these serious infections.

We defined a standard treatment duration of 10 days recognizing that sometimes bacteremia, especially that with a removable focus, can be safely treated with less than 14 days. For antibiotic

stewardship purposes, there is an interest in shortening treatment duration especially with antibiotics such as colistin and meropenem. We did not want to impose prolonged treatment durations in the study centers for this reason. However, we encourage treatment duration as necessary beyond the standard duration.

2. Clarify infusion time of meropenem dosing for Crcl < 50.

Clarification was added in the text: “For patients with impaired renal function, dosing is adjusted (Table 2) without a change in the infusion time [3 hours]”.

3. I would recommend additional colistin PK sampling. (current reference is 30, but I think you meant 31) The garzonik study collected samples on day 3 or 4, based on the long half-life. Consider obtaining levels on day 3 or 4.

The number of samples for drug concentration measurement was carefully considered based on cost, practical aspects and the additional information that an additional time point would contribute, given that we have already characterized the PK of CMS and colistin in similar patient populations [1,2]. Since we intend to analyze relationships between covariates such as CrCL and weight with colistin exposure (determined primarily by the pharmacokinetic parameter apparent colistin clearance) and explore the exposure-response relationships we prioritized few samples per patient, but sampling in all patients, over an extended sampling schedule in fewer patients. The sampling schedule was carefully estimated using optimal experimental design theory, considering that individual exposure should be estimated with as low uncertainty as possible. The methodology for the optimal design is now available in a published article [3]. Sampling up to 72 h was allowed in the estimation of the optimal sampling time points, but sampling during the first 24h was determined to provide most information on the individual parameters. Sampling during e.g. day 3 would contribute with less information on the inter-patient vs. the inter-day variability in colistin exposure, as well as carry limited information on the volume of distribution. Moreover, early sampling reduces the risk that sampling is affected by dropout which could potentially lead to bias in which patients who had the colistin exposure determined. An intermediate evaluation of the design has been performed using collected and analyzed samples for the first 100 recruited patients [see thesis by Anders Kristoffersson https://uu.diva-portal.org/smash/get/diva2:862010/FULLTEXT01.pdf]. This analysis shows similar uncertainty in individual parameters as expected based on the optimal design.

Evelyn Shaw Perujo

4. Key words: the study is focused on carbapenem-resistant Gram negative infections and Key words section doesn’t include this word. They used multidrug resistant gram negative bacteria which is not exactly the same. Maybe will be better change the word to carbapenem-resistant?

The keyword was changed as suggested.

5. Abstract: primary outcome says is a composite variable assessed at day 14: survival + hemodynamic stability + clinical stability “or” improvement. But in outcomes section the primary outcome is defined as patient alive at day 14 + hemodynamic stability + “and” improvement of SOFA. I think given the strong interest of the study that the definition of primary outcome has to be strictly defined, it should not allow different interpretation of data. Again, as I said in the inclusion/exclusion criteria section, to list the outcomes followed of a definition section could help a better understanding of this section. Currently is a little bit confused.

The primary outcome is meant to include stability or improvement of the SOFA score. A correction was made to the abstract.

6. Objectives and Trial design: the authors wrote, the primary objective is to show superiority..... in the treatment of patients infected with MDR GNB. It will be better change MDR GNB per Carbapenem- resistant GNB which is the aim of the study.

The change was made.

7. Objectives and Trial design: objectives should be defined more accurately. How researchers have defined the concept of “superiority” in the study? How is planned to be measured, Any specific outcome?. They are interested in any other clinical or microbiological objectives related to the concept of “superiority”. The authors also wrote just one secondary objective related to pharmacokinetic models of colistin. This means they are going to measure Colistin steady-state plasma concentrations. To define “the specific objectives” is recommended for a better understanding of the study

We debated on the optimal outcome and tried to compose an outcome that will reflect cure of infection and patients’ wellbeing as fully as possible. The first and most important is obviously survival. Then, among surviving patients we thought that hemodynamic and vital organ system failure stability or improvement would most be reflective of antibiotics’ ability to resolve the infection. Regarding the time point we selected (14 days), we wanted a time point as far away from infection onset as possible since for patients the longer perspective is more relevant, but given the severe baseline illnesses of included patients with carbapenem-resistant Gram-negative infections, we deemed it inappropriate to rely on a time point after 2 weeks to avoid dilution of the differences between arms by deaths unrelated to infection and subsequent infections. The advantages of the outcome are that it was designed by clinicians caring for such patients addressing the patient perspective. The limitation is that it was not used previously so that we cannot compare our outcome rates to other studies. We defined superiority as improvement of 15% in this composite measure. The specific outcomes of the study including definitions and means of assessment are fully explained in the section “Outcomes”. The definition of superiority is provided in the same size calculation section. Please note that we are not measuring colistin steady-state concentrations, but based on the measurements we can predict the steady-state concentrations. See also the reply to item number 3.

8. The inclusion/exclusion criteria will be easier to understand if they be listed. Currently the section of inclusion criteria includes exclusion criteria!. Definitions should be written in a section apart (as it is for example table 1).
Does the study include within the inclusion criteria the signed informed consent? And pregnancy test in fertile women?

The inclusion and exclusion criteria have been rewritten for greater ease of understanding. Informed consent is an inclusion criterion and has been stated explicitly as such. Routine pregnancy testing of presumably fertile women unknown to be pregnant will not be conducted as part of the study and this has been addressed in the body of the text. The population of patients we encounter with carbapenem-resistant bacteria makes pregnancy very unlikely.

9. Table 1 includes UTI definition which is not the current definition of cUTI, one of the populations in this study. Which disease is wanted to be included, UTI or cUTI?.

Our definition of cUTI is intended to address sepsis in which the presumed source of infection is the urinary tract. The focus was on septic patients, so as not to include patients with asymptomatic

bacteriuria. We changed the term to urosepsis, concordant with Kunin's suggestion to term this group of patients as "urosepsis syndrome" or "complicated urinary tract infection with sepsis" [4]. The text has been amended for clarification.

10. To get an easier understanding it would be better to separate in the intervention section: arm 1 colistin /arm 2 Colistin + meropenem. Dot apart, put the duration of therapy and antibiotics permitted and not permitted.

The section has been rewritten as suggested.

11. The study contemplate criteria for discontinuing or modifying allocated interventions for a given trial participant?

Patients enrolled in the study have an infection with an isolate for which colistin is the drug of choice. As the purpose of the RCT is to determine whether the combination of meropenem + colistin is superior to colistin alone, modification of allocation would threaten to nullify or blur the distinction between the regimens. Therefore, we preferred to discourage treatment modifications and did not define protocol criteria for discontinuation/ modification. We left decisions to the caretaking physicians although we stress the importance of attempting to maintain the assigned treatment for at least 5 days unless something critical happens. In the primary analysis we will account for all patients as randomized in the intention to treat principal recommended for superiority trials. In a per protocol analysis, we will exclude patients that did not receive the allocated intervention for at least 5 days. This is defined in the protocol.

12. Outcome section: in Abstract section, primary outcome says "is a composite variable assessed at day 14: survival + hemodynamic stability + clinical stability "or" improvement". But in outcomes section the primary outcome is defined as patient alive at day 14 + hemodynamic stability + "and" improvement of SOFA. I think, given the high interest of this study, the definition of primary outcome has to be strictly defined, it should not allow different interpretation of the data.

Again, as I said in the inclusion/exclusion criteria section, listing the outcomes followed by a definition section could help a better understanding of this section. Currently the section is written in a very confused way.
It will be also appreciated specify in simpler way, what is considered success and what is failure in the study.

We agree. The abstract has been modified (see response above). The outcomes section and accompanying table have been rewritten. Clearer definitions of success and failure have been provided.

13. Timeline for follow up: I understand all patients will be followed during 28 days, it does not depend on total duration of therapy?. I understand table 4 includes visits for participants? Could it be better specified? Maybe as an appendice and just include a summary in the text.

Yes, all patients are followed for 28 days or until death if earlier. Clarification has been provided in the text: "All patients will be followed up to 28 days following enrollment in the trial. For hospitalized patients, follow-up will be performed on a regular basis through study visits (Table 4) and daily through patients' electronic records. For the rare instances in which patients are discharged before day 28, follow-up will be completed via the appropriate healthcare system databases."

14. How is expected to recruit the patients? How they will be identified in each hospital?

Patients are identified from daily or twice-daily reports from the microbiological laboratories on CRGNB isolates identified in the lab from blood, urinary or sputum cultures. A study investigator assesses the clinical relevance of the isolate and applies inclusion and exclusion criteria. This information has been added to the text.

15. Data collection and microbiological sampling: I'm concerned because authors put in the document "other samples are obtained as clinically indicated". What does this means?. A clinical trial should have very well defined variables to allow all the investigators to do things in the same way.

The intention is to address microbiological sampling taken by treating physicians unassociated with the study. We have the trial protocol for all procedures required for the trial, including patient monitoring, laboratory and microbiological sampling. Beyond that physicians were allowed to take samples as needed. The text has been amended for clarification.

16. Statistical analysis: significance is considered only if p= 0.05 or ≤ 0.05?

p<0.05. Thanks, corrected.

17. Safety monitoring: Is there any adverse event of particular interest in the study?. If yes, specify please.

Meropenem is a relatively safe drug that is in common use in hospitals. Its addition to colistin is not expected to result in unknown adverse events. The main concern with the addition of meropenem is further resistance maintenance and induction of resistance. Randomized controlled trials are not the optimal or appropriate platform to address effects of antibiotics on resistance, as the time frame for resistance development and detriments are longer than the follow-up duration of a clinical trial and perhaps resistance development should be a assessed at the unit level rather than at the individual level. However, we will try to examine outcomes related to resistance development in the individual level: we will examine for gastrointestinal carriage of carbapenem and colistin-resistant bacteria throughout and after treatment (through the weekly rectal surveillance swabs), we document all such clinical isolates identified in samples actively taken for the trial and in those taken by clinicians. Another important adverse event we will monitor is Clostridium difficile infection. We added a brief statement of this in the safety monitoring section

18. Discussion: There is a huge discussion related to "Ethics" and I'm not sure if it's needed in this section. However, I think the final decision is on the Editor

Due to specific ethical issues of relevance in the patient population being studied we felt it was important to provide a detailed description of the means for obtaining informed consent. Our trial raises important issues in the informed consent process of comparative effectiveness studies assessing interventions that are interchangeable in clinical practice. Especially given the study population and in difficulty of other ongoing trials in this patient population to recruit patients. We will appreciate if we can keep the ethical discussion in the manuscript. However, we shortened this part in the methods section to avoid repetition and we rephrased in discussion to clarify the importance of this discussion to studies on carbapenem-resistant GNs.

References

1. Plachouras D, Karvanen M, Friberg LE, et al. Population pharmacokinetic analysis of colistin methanesulfonate and colistin after intravenous administration in critically ill patients with infections

caused by gram-negative bacteria. Antimicrob Agents Chemother 2009;53:3430-6.
2. Mohamed AF, Karaiskos I, Plachouras D, et al. Application of a loading dose of colistin methanesulphonate in critically ill patients: population pharmacokinetics, protein binding and prediction of bacterial kill. Antimicrob Agents Chemother 2012;56:4241-9.
3. Kristofferson AN, Friberg LE, Nyberg J. Inter occasion variability in individual optimal design. J Pharmacokinet Pharmacodyn 2015;42:735-50.
4. Kunin CM. Definition of acute pyelonephritis vs the urosepsis syndrome. Arch Intern Med 2003;163:2393.

VERSION 2 – REVIEW

REVIEWER	Evelyn Shaw Perujo Hospital Universitari de Bellvitge-IDIBELL. Hospitalet de Llobregat. Barcelona. Spain
REVIEW RETURNED	27-Feb-2016

GENERAL COMMENTS	Very good and interesting study. I'll be happy to read the results.