PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Multicentre, randomised, open-label, phase IV-III study to evaluate the efficacy of cloxacillin plus fosfomycin versus cloxacillin alone in adult patients with methicillin-susceptible Staphylococcus aureus bacteraemia: study protocol for the SAFO trial.
AUTHORS	Grillo, Sara; Cuervo, Guillermo;Carratala, Jordi; San-Juan, Rafael; Aguado, Jose; Morata, Laura; Gomez-Zorrilla, Silvia; López-Contreras, Joaquín; Gasch, Oriol; Gomila-Grange, Aina; Iftimie, Simona; Garcia-Pardo, Graciano; Calbo, Esther; Boix-Palop, Lucía; Oriol, Isabel; Jover-Sáenz, Alfredo; López-Cortés, Luis Eduardo; Euba, Gorane; Aguirregabiria, Malen; Garcia-Pais, Maria Jose; Gioia, Francesca; Paño, Jose Ramón; Pedro-Botet, Maria Luisa; Benítez, Rosa Maria; Pérez-Rodríguez, Maria Teresa; Meije, Yolanda; Loeches-Yagüe, Maria Belén; Horna, Gertrudis; Berbel, Damaris; Domínguez, Maria Ángeles; Padullés, Ariadna; Cobo, Sara; Hereu, Pilar; Videla, Sebastian; Tebe, Cristian; Pallarés, Natàlia; Miro, Josep; Pujol, Miquel

VERSION 1 – REVIEW

REVIEWER	Venditti, Mario Sapienza University of Rome, Sto arrivando!nità Pubblica e M infettive
REVIEW RETURNED	02-Apr-2021

GENERAL COMMENTS	First of all I would congratulate the authors for investigating an important topic as the one of potential efficacy of combination therapy with adjunctive fosfomycin for MSSA BSI. The study protocol appears rigorous and the opportunity of a multi center investigation supports generalisability of the results. Indeed, I have no major suggestions for substantial improvement of the study protocol. Minor comments: - pag 11 line 153 and pag 15 lines 258-9. I am convinced of the presence of a synergistic interaction between betalactams and fosfomycin against S aureus; however, to my knowledge there is no mention in references 14,15,16 of the presence of in vitro synergism between cloxacillin (.. and oxacillin, flucloxacillin or cefazolin) against MSSA but only against MRSA. Thus I concur with the authors that this study provides an excellent opportunity to further address this issue as well as the relationship between in vitro synergism and clinical outcome in MSSA BSI. To this end

	details of methods of fosfomycin MIC determination and in vitro synergy studies would be presented. - pag 15 line 264. Just for the usual reader go BMJ that might be not an ID specialist I would suggest to explain in few words the rationale of of investigating the relationship between high Vanco/Dapto MICs and MSSA virulence.

REVIEWER	Brade, Karrine Boston Medical Center
REVIEW RETURNED	11-Apr-2021

GENERAL COMMENTS	In the introduction, it is stated that there is no optimal treatment for MSSA bacteremia. This is not entirely true, given that monotherapy with beta-lactams has been shown to reduce mortality compared to other therapies (vancomycin) and they are considered the standard of care. I think the more accurate statement is whether combination therapy could reduce duration of bacteremia or reduce mortality compared with the current standard of care (monotherapy beta-lactams). I would recommend re-wording this section slightly to address this change. In the treatment success at TOC visit definition, no isolation of MSSA in blood culture 'or at another sterile site' - clarify 'and/or' another sterile site?

REVIEWER	Lee, Todd McGill University Health Centre
REVIEW RETURNED	22-Apr-2021

GENERAL COMMENTS	This is an exciting trial to read and the authors should be commended. It appears that the study has been started in 2019 on clinicatrials.gov and it is recruiting; therefore, I would imagine that there is limited opportunity for peer review to change any major aspects protocol. In that case, I suppose the comments may serve as early peer review for the final product. They are meant to be helpful comments and/or observations and not harsh criticisms. Abstract: Concise. Most specific comments will appear in the main text. Strengths and limitations We don't know that cloxacillin is the best treatment for MSSA bacteremia – it may turn out that cefazolin is as good or superior. I would suggest removing "offer the best antibiotic treatment for MSSA bacteremia and to" I agree open label is a potential problem. I think you should talk about how you will use methods to account for cross-over in your analysis. For instance, I think it is much more likely that someone who is assigned to no fosfo will get fosfo added on than for fosfo to be discontinued (outside of major toxicity). Introduction

	Line 123: please cite this. I thought that the expected rates for MSSA were lower than that, but I could be thinking about 30 days so also include the time point. Line 126: There is some debate as to what constitutes complicated MSSA bacteremia, but I think it is generally more than the definition you provide. For instance: https://jamanetwork.com/journals/jamainternalmedicine/fullarticle/216060 gives one commonly used definition (but there are others). Line 132: Reverse the comparison? --- nor is it clear whether combination therapy is better than monotherapy. Monotherapy is the current standard which you are comparing to. Line 133: The Americans don't have cloxacillin. They have nafcillin. The Australians have flucloxacillin. So, the standard treatment is really monotherapy with an anti-staphylococcal penicillin. You haven't mentioned cefazolin, which is also commonly used. It may be reasonable to discuss why cefazolin can't be the standard of care and why cefazolin isn't in your study. Realistically, cefazolin plus fosfomycin could be the best regimen because you get less toxicity than cloxacillin with the fosfomycin countering any possible inoculum effect. Line 149: Please cite evidence for the statement that monotherapy leads to resistance. Line 154: Since this trial is about mortality, I think you should address any mortality difference in your trial. Is the reader to infer that "younger severely ill patients" is a subgroup of your trial result? Or were those the population of patients you recruited? If it is a subgroup, this should be made more transparent. Line 156: Suggest change to no other randomized studies. Yours is in the clinical trials database already. Methods Line 166: What is a phase IV-III trial? Line 191: Why are you excluding pregnant and breastfeeding women? This should be justified given an international focus on not excluding these patients from clinical research if at all possible. Line 202: Could you provide a citation for the requirement to dose adjust cloxacillin below 30mL/minute? We do not dose adjust here in Canada. What formula are you using to calculate the clearance? Are you adjusting clearance for obesity? Line 208: Please provide explicit definitions for uncomplicated and complicated bacteremia. Line 215: You should also report a per protocol (as per CONSORT). Line 220: Day 7 as calculated from when? The time of the initial culture? Of first receipt of study drug? Another day 1? This should be explicit. (Note: I see it is explicit in the figure, but it should be in the text too)

	Line 227: So, the TOC visit is the maximum of 12 weeks after randomization or 2 weeks after end of therapy? Line 236: What does “lack of clinical improvement” mean? Line 242: So, these are after randomization? In this case, it becomes very important that the two arms are balanced between the # of days PRIOR to randomization. Because if you get enrolled at 71 hours, +3 days has a very different meaning than enrolled at 24 hours + 3 days. Line 249: Not sure that endocarditis will be prevented by fosfomycin? I would imagine that if a patient has endocarditis from MSSA bacteremia, they probably have it at presentation and do not develop it de novo on therapy. Likewise, the patient has a prosthetic on presentation most of the time, not after the bacteremia (except perhaps in bad endocarditis, discitis or other infection which needs urgent OR). I do agree you need to capture this data, but I am not sure it belongs as an outcome of the trial. Line 251, 252: This will need to be adjusted for the competing risk of death. If you are dead, you can accrue no more days of stay/treatment. Line 253: Subgroup analyses for which of the primary outcomes? Both? Isn’t persistent bacteremia at day 7 part of your primary outcome? Line 276: Please explicitly provide the safety outcomes in an appendix. Line 299: Are we speaking about your day 7 primary outcome? This should be explicit. If so, you are suggesting that the rate of the day 7 composite outcome with monotherapy is expected to be 74%. Did you account for lag to randomization and the differences between what would be day 1 in your observational study and day 1 in your RCT? I worry you will find a higher rate of success because you are (a) excluding people at high risk of death and (b) the conditional survival of enrolled patients will be higher because they have lived long enough to enroll. Further, they will all have received 1-3 days or pre-treatment before day of randomization. That said, if the drug is really 12% absolute more effective, you will still have adequate power. I do not see a sample size or power estimates for your co-primary outcome? How will you spend alpha between the 2 primary outcomes? Am I correct from reading below that you will only test additional hypotheses if the day 7 outcome is positive? What if the day 7 outcome is a bad proxy for week 12 outcomes and you have a positive week 12 outcome but a negative day 7 outcome? Are there interim analyses? If so, how will alpha be spent? How will you analytically deal with cross-over from no fosfomycin to fosfomycin in this open label study? I am specifically concerned that patients are more likely to go from clox -> clox + fosfo than the other

	way around and this will bias towards the null if clox+fosfo is indeed better. Analytically, by ITT you'll need to keep this in the clox assignment but I wonder if there is a way you will get around this risk? Line 316: I think that CONSORT suggests you should also report an absolute risk difference and 95% confidence interval. Line 331: What criteria will the DSMB use to decide to continue/terminate? For efficacy and for futility/harm? Can be in appendix Line 351: Do you think that the open label nature of this study might lead to excess furosemide use in the combination arm? Are you talking about "prophylactic" furosemide or they will get furosemide if there is clinical evidence of congestive heart failure or other volume overload? Line 372: current local legislation. Line 373: duplicative to line 372 Overall, you could tighten up the ethics section a bit I think (make it more concise). Figure 1 makes the day# much more explicit. This should be clarified in the text.

VERSION 1 – AUTHOR RESPONSE

Reviewer #1
Dr. Mario Venditti, Sapienza University of Rome
Comments to the Author:
First of all I would congratulate the authors for investigating an important topic as the one of potential efficacy of combination therapy with adjunctive fosfomycin for MSSA BSI. The study protocol appears rigorous and the opportunity of a multi center investigation supports generalisability of the results.
Indeed, I have no major suggestions for substantial improvement of the study protocol.
Minor comments:
- pag 11 line 153 and pag 15 lines 258-9. I am convinced of the presence of a synergistic interaction between betalactams and fosfomycin against S aureus; however, to my knowledge there is no mention in references 14,15,16 of the presence of in vitro synergism between cloxacillin (.. and oxacillin, flucloxacillin or cefazolin) against MSSA but only against MRSA. Thus I concur with the authors that this study provides an excellent opportunity to further address this issue as well as the relationship between in vitro synergism and clinical outcome in MSSA BSI. To this end details of methods of fosfomycin MIC determination and in vitro synergy studies would be presented.
Response:
We truly agree with the reviewer, so we have modified the sentence accordingly (line 155 of the tracked version).
Furthermore, we have added the methodology for fosfomycin MIC determination and in vitro synergy studies in supplementary material.
- pag 15 line 264. Just for the usual reader go BMJ that might be not an ID specialist I would suggest to explain in few words the rationale of investigating the relationship between high Vanco/Dapto MICs and MSSA virulence.
Response:
As suggested, we have explained the rationale (lines 273-276 tracked version).

Reviewer #2
Dr. Karrine Brade, Boston Medical Center
Comments to the Author:
In the introduction, it is stated that there is no optimal treatment for MSSA bacteremia. This is not entirely true, given that monotherapy with beta-lactams has been shown to reduce mortality compared to other therapies (vancomycin) and they are considered the standard of care. I think the more accurate statement is whether combination therapy could reduce duration of bacteremia or reduce mortality compared with the current standard of care (monotherapy beta-lactams). I would recommend re-wording this section slightly to address this change.
Response:
We truly agree with the reviewer, thus we have changed the sentence as suggested (lines 131-132 of tracked version).

In the treatment success at TOC visit definition, no isolation of MSSA in blood culture 'or at another sterile site' - clarify 'and/or' another sterile site?
Response:
As suggested, we added “and/or” in the sentence (Line 235 of tracked version).

Reviewer #3
Dr. Todd Lee, McGill University Health Centre
Comments to the authors:
This is an exciting trial to read and the authors should be commended. It appears that the study has been started in 2019 on clinicatrials.gov and it is recruiting; therefore, I would imagine that there is limited opportunity for peer review to change any major aspects protocol. In that case, I suppose the comments may serve as early peer review for the final product. They are meant to be helpful comments and/or observations and not harsh criticisms.
Abstract: Concise. Most specific comments will appear in the main text.
1. Strengths and limitations. We don’t know that cloxacillin is the best treatment for MSSA bacteremia – it may turn out that cefazolin is as good or superior. I would suggest removing “offer the best antibiotic treatment for MSSA bacteremia and to”.
Response:
We modified the sentence as suggested (Lines 107-108).

2. I agree open label is a potential problem. I think you should talk about how you will use methods to account for cross-over in your analysis. For instance, I think it is much more likely that someone who is assigned to no fosfo will get fosfo added on than for fosfo to be discontinued (outside of major toxicity).
Response:
We agree that cross-over is a limitation, inherent to the open label design. Up to date, no clinical evidence supports the use of fosfomycin in MSSA bacteraemia. In fact, according to clinical guidelines, adding a second antibiotic to the standard of care is not indicated.
In our study, crossovers between study arms are considered as “treatment failure” (both if fosfomycin is added in the monotherapy arm as well as if it is stopped because of toxicity in the combination therapy arm).

3. Introduction Line 123: please cite this. I thought that the expected rates for MSSA were lower than that, but I could be thinking about 30 days so also include the time point.
Response:
We added the time point (90 days) and a reference (lines 123-124 of tracked version).

4. Line 126: There is some debate as to what constitutes complicated MSSA bacteremia, but I think it is generally more than the definition you provide. For instance: https://jamanetwork.com/journals/jamainternalmedicine/fullarticle/216060 gives one commonly used definition (but there are others).
Response:
We agree with the reviewer. We have removed this incomplete definition from the introduction (lines 126-127 of tracked version) and we provide a more accurate definition for complicated and uncomplicated bacteremia in methods (Lines 211-215 of tracked version). We also added a reference at the end of the sentence.

5. Line 132: Reverse the comparison? --- nor is it clear whether combination therapy is better than monotherapy. Monotherapy is the current standard which you are comparing to.
Response:
We modified the sentence as follows: “it is not clear whether combination therapy could reduce duration of bacteremia or reduce mortality compared with the current standard of care (beta-lactam monotherapy)”.

6. Line 133: The Americans don’t have cloxacillin. They have nafcillin. The Australians have flucloxacillin. So, the standard treatment is really monotherapy with an anti-staphylococcal penicillin. You haven’t mentioned cefazolin, which is also commonly used. It may be reasonable to discuss why cefazolin can’t be the standard of care and why cefazolin isn’t in your study. Realistically, cefazolin plus fosfomycin could be the best regimen because you get less toxicity than cloxacillin with the fosfomycin countering any possible inoculum effect.
Response:
We agree with your comment and thus we have changed “cloxacillin” in line 135 (tracked version). Regarding cefazolin, unfortunately, we have already started recruitment of patients and we believe that changing the backbone therapy at this point could implicate a relevant modification of study protocol, which we consider inappropriate at this time with the recruitment ongoing.

7. Line 149: Please cite evidence for the statement that monotherapy leads to resistance.
Response:
We added a reference in line 153, as suggested.

8. Line 154: Since this trial is about mortality, I think you should address any mortality difference in your trial. Is the reader to infer that “younger severely ill patients” is a subgroup of your trial result? Or were those the population of patients you recruited? If it is a subgroup, this should be made more transparent.
Response:
In this line we are talking about the results of a previous published trial [1]. However, we agree with the reviewer that this planned sub-analysis should be more transparent, so we have specified the subgroup in line 158 of tracked version.

9. Line 156: Suggest change to no other randomized studies. Yours is in the clinical trials database already.
Response:
We have changed the sentence as suggested (line 160 of tracked version).

10. Methods Line 166: What is a phase IV-III trial?
Response:

A phase IV-III trial is considered a trial involving commercialized drugs (phase IV) but with a new use (phase III) compared with the previous indications. In the SAFO trial, we considered combination treatment as a new use of these antibiotics.

11. Line 191: Why are you excluding pregnant and breastfeeding women? This should be justified given an international focus on not excluding these patients from clinical research if at all possible.
Response:
Despite the relative safety profile of cloxacillin and fosfomycin, we believe that combination could bring a sodium overload that should be avoided in pregnant women, particularly at the end of pregnancy. Moreover, physiological changes during pregnancy may result in changes to drug plasma levels and could bring to associated dose-related adverse reactions or under-treatment, either of which could have negative consequences on the pregnancy outcome. For drugs excreted in breastmilk, there could be a risk of immediate adverse event in the child and a risk of accumulation in the infant, so we prefer to exclude this population from the study [2].

12. Line 202: Could you provide a citation for the requirement to dose adjust cloxacillin below 30mL/minute? We do not dose adjust here in Canada. What formula are you using to calculate the clearance? Are you adjusting clearance for obesity?
Response:
We considered different dosage recommendations:
1. Product Information of Anaclosil® recommends to reduce the daily dose to 50% the standard dose when CLCR<30 mL/min. https://cima.aemps.es/cima/pdfs/es/ft/55418/55418_ft.pdf
2. Product Information of Cloxacillin Normon® recommends dose adjustment in patients with renal impairment. https://cima.aemps.es/cima/pdfs/es/ft/63636/63636_ft.pdf
3. According to “Kucers' The Use of Antibiotics” book, in the presence of severe renal impairment, the dosage of cloxacillin should generally be reduced, especially if very high parenteral doses are used. (Kucers' The Use of Antibiotics. A Clinical Review of Antibacterial, Antifungal, Antiparasitic, and Antiviral Drugs, 7th edition).
4. Azanza et al recommends 0.5-1g/12h when CLCR is 10-50 ml/min (Enferm Infecc Microbiol Clin. 2009;27(10):593–599).
We calculate creatinine clearance from serum creatinine concentrations according to Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula. We do not adjust clearance for obesity.

13. Line 208: Please provide explicit definitions for uncomplicated and complicated bacteremia.
Response:
Please see our response to your previous question (question 4).

14. Line 215: You should also report a per protocol (as per CONSORT).
Response:
As suggested, we have clarified this issue (Line 223 of tracked version).

15. Line 220: Day 7 as calculated from when? The time of the initial culture? Of first receipt of study drug? Another day 1? This should be explicit. (Note: I see it is explicit in the figure, but it should be in the text too)
Response:
We calculate 7 days from randomization. As suggested, we have clarified this time-point definition in the main text (Line 226 of tracked version).

16. Line 227: So, the TOC visit is the maximum of 12 weeks after randomization or 2 weeks after end of therapy?
Response:
The TOC visit will be performed at 12 weeks after randomization. Only in those patients needing a more prolonged therapy (e.g. 14 weeks), the TOC visit will be performed 2 weeks after the EOT (in the e.g., 16 weeks from randomization).

17. Line 236: What does “lack of clinical improvement” mean?
Response:
Lack of clinical improvement is considered when there is no “clinical improvement measured by stable or improved quick SOFA score compared with baseline”, as stated in line 229.

18. Line 242: So, these are after randomization? In this case, it becomes very important that the two arms are balanced between the # of days PRIOR to randomization. Because if you get enrolled at 71 hours, +3 days has a very different meaning than enrolled at 24 hours + 3 days.
Response:
Endpoints will be assessed at the indicated time-points after randomization. We agree with the reviewer that patients could be randomized early or late within the time window of randomization. This relevant difference will be considered at the moment of the statistical analysis.

19. Line 249: Not sure that endocarditis will be prevented by fosfomycin? I would imagine that if a patient has endocarditis from MSSA bacteremia, they probably have it at presentation and do not develop it de novo on therapy. Likewise, the patient has a prosthetic on presentation most of the time, not after the bacteremia (except perhaps in bad endocarditis, discitis or other infection which needs urgent OR). I do agree you need to capture this data, but I am not sure it belongs as an outcome of the trial.
Response:
We agree with the reviewer. Most patients could have endocarditis at presentation. We consider important to collect this information, although we agree that there is not necessarily a causal relationship between this event and the study treatment.

20. Line 251, 252: This will need to be adjusted for the competing risk of death. If you are dead, you can accrue no more days of stay/treatment.
Response:
As suggested, we considered this adjustment in the statistical analysis plan (line 333-334 of tracked version).

21. Line 253: Subgroup analyses for which of the primary outcomes? Both? Isn’t persistent bacteremia at day 7 part of your primary outcome?
Response:
We will perform exploratory subgroup analyses for patients at high risk (those with metastatic infection, unknown focus of bacteremia, endocarditis, and pneumonia) for both primary outcomes. On participants with persistent bacteremia, subgroup analysis will be focused on treatment success at TOC (we have updated this information in Line 259 of tracked version).

22. Line 276: Please explicitly provide the safety outcomes in an appendix.
Response:
We agree with the reviewer and we added the safety outcomes in Supplementary material.

23. Line 299: Are we speaking about your day 7 primary outcome? This should be explicit. If so, you are suggesting that the rate of the day 7 composite outcome with monotherapy is expected to be 74%. Did you account for lag to randomization and the differences between what would be day 1 in your observational study and day 1 in your RCT? I worry you will find a higher rate of success because you are (a) excluding people at high risk of death and (b) the conditional survival of enrolled patients will be higher because they have lived long enough to enroll. Further, they will all have received 1-3 days or pre-treatment before day of randomization. That said, if the drug is really 12% absolute more effective, you will still have adequate power. I do not see a sample size or power estimates for your co-primary outcome? How will you spend alpha between the 2 primary outcomes? Am I correct from reading below that you will only test additional hypotheses if the day 7 outcome is positive? What if the day 7 outcome is a bad proxy for week 12 outcomes and you have a positive week 12 outcome but a negative day 7 outcome? Are there interim analyses? If so, how will alpha be spent? How will you analytically deal with cross-over from no fosfomycin to fosfomycin in this open label study? I am specifically concerned that patients are more likely to go from clox -> clox + fosfo than the other way around and this will bias towards the null if clox+fosfo is indeed better. Analytically, by ITT you'll need to keep this in the clox assignment but I wonder if there is a way you will get around this risk?
Response:
Sample size has been calculated on expected mortality at TOC (12 weeks after randomization). It is true that we could overestimate mortality at the TOC visit. For this reason, we have planned an interim analysis that will be performed when half part of the patients will be included. The aim of this interim analysis is to ensure the correct progress of the study in terms of safety, and also to check the appropriateness of the sample size assumptions (lines 339-346 of tracked version). No efficacy analysis will be performed in this interim analysis. The data monitoring board committee will give a recommendation to the sponsor concerning the continuation of the study or sample size adjustments.

24. Line 316: I think that CONSORT suggests you should also report an absolute risk difference and 95% confidence interval.
Response:
We agree with the reviewer. As suggested, we added the sentence "Absolute risk difference and 95% confidence interval will also be reported" in Line 331 of the tracked version.

25. Line 331: What criteria will the DSMB use to decide to continue/terminate? For efficacy and for futility/harm? Can be in appendix
Response:
The data monitoring board will ensure the correct progress of the study in terms of safety, and also the sample size assumptions. The review by the DSMB will be performed when half of the sample size will be reached. (See lines 339-346 of tracked version).

26. Line 351: Do you think that the open label nature of this study might lead to excess furosemide use in the combination arm? Are you talking about "prophylactic" furosemide or they will get furosemide if there is clinical evidence of congestive heart failure or other volume overload?
Response:
We assume that the combination arm will receive most frequently furosemide. Our advice is to prescribe oral low dose "prophylactic" furosemide and to adjust the dose based on clinical daily examination.

27. Line 372: current local legislation.
Response:
We have modified the sentence (Line 387 of tracked version).

28. Line 373: duplicative to line 372 Overall, you could tighten up the ethics section a bit I think (make it more concise). Figure 1 makes the day# much more explicit. This should be clarified in the text.
Response:
We deleted the duplicated lines (388-390 of the tracked version). Furthermore, we made the Ethics section more concise and moved part of data patients' management to the Supplementary Material.
As previously explained, we have clarified in the main text the days from randomization (Line 226 of tracked version).

VERSION 2 – REVIEW

REVIEWER	Lee, Todd McGill University Health Centre
REVIEW RETURNED	18-Jun-2021

GENERAL COMMENTS	Thank you for providing these revisions which have clarified the minor point. Good luck with the trial we are all excited to see the results.

VERSION 2 – AUTHOR RESPONSE

Reviewer: 3

Dr. Todd Lee, McGill University Health Centre

Reviewer: 3

Competing interests of Reviewer: I am a principal investigator on another RCT involving MSSA bacteremia.