Reviewer’s report

Title: A randomized, double-blind clinical phase II trial of the efficacy, safety, tolerability and pharmacokinetics of a single dose combination treatment with artefenomel and piperaquine in adults and children with uncomplicated Plasmodium falciparum malaria

Version: 0 Date: 29 May 2017

Reviewer: Kasia Stepniewska

Reviewer's report:

Major

1. Methods section required a little of re-writing and restructuring, in particular:

Definition of Endpoints should come before statistical consideration; Definitions are mixed up with methods of analysis (for example for K13 and PCT1/2); Important details of methodology should be given, not just referred to (as it is difficult to find them): parasite counting, PCR genotyping, pharmacological sampling.

2. Efficacy analysis

a) It should be clearly stated how many recrudescences and new infection were recorded at each of the weekly visits. For ACPR calculation and for Kaplan-Meier analysis, it should be clearly stated how patients with new infections were treated.

b) Figure 2. Please state what are the numbers at the top of the whiskers

c) Figure 3. Censored observations and numbers at risk should be presented. It is especially important that for some of the patients duration of follow-up was only 42 days. Y-axis could be truncated to 0.6 so that the curves are better visible

d) Page 20. Lines 379 - 381. These statements are too vague. Please see earlier comment regarding number of recurrences, recrudescences and new infections. If presenting the recurrence rate, 95% CI should also be provided and the time of evaluation (i.e 28 days?)
3. K13 and PCT1/2 analysis

a) Line 389. Median percentage - it is unclear from what values it is calculated. Isn't this just a proportion of patients who cleared parasite by 72 hours?

b) Line 391. How was the dose-response relationship explored? Perhaps statement like this would be sufficient "there were no significant differences in proportion of patients achieving parasite clearance by 72 hours between treatment arms or age groups in Africa."

c) Line 394. How was X% parasite clearance calculated? Could you also present range for X% parasite clearance, it is especially interesting if there are subgroups of patients with delayed clearance.

d) Lines 398-400. More details should be given for the estimation of PCT1/2 - ranges, goodness of fit. It is unclear what "within Vietnam, PCT1/2 was similar across study centre" means.

e) All K13 mutations detected should be listed.

f) Figure 4. Could you present all K13 mutations on x-axis? There are few patients with PCT1/2 >10 hours - can you confirm if the model fit was appropriate for these patients. There is a significant number of patients in Africa with HL> 5 hours. This seems like slower clearance than in patients treated with standard ACTs (see for example https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4578675/). Did you check goodness of fit for these patients? Can you comment why this could be?

g) Line 412-413. The last sentence does not belong here but in section Exposure-Response relationship.

4. Safety and Tolerability.

a) Timing of TEAE/TESAE should be included where relevant.
b) Table 5 could be omitted - first row is repeated in Table 6, most of the remaining information is given in text which could be slightly expanded to cover all information from Table 5.

5. From figures presented in supplementary material S3, I am guessing that piperaquine and artefenomel concentrations were measured on day 7 in all patients. Were these concentrations also evaluated against the outcome? It is unclear if these concentrations were analysed at any stage, or if only predicted concentrations were used. If they are indeed available, can you compare values of the observed and predicted concentrations. If the observed day 7 concentrations were not used in the response model, can you perform sensitivity analysis by fitting model with the observed values.

In several places it is unclear if you are referring to the observed or predicted concentrations (for example: Page 12. Line 282; Figure 8)

6. Exposure-Response Analysis

a) Have you considered running survival regression model instead of logistic model. It has an advantage of being able to include all patients who did not complete the final visit and takes into account the time of recrudescence which is relevant when exploring effect of the concentration at day 7. This analysis may give you more power to explore effects of covariates.

b) Figure 6. Caption - it should be clear that these are predicted concentrations and logistic model predictions are shown. Can you also provide number of patients in each bin.

c) In Table 4, 340 patients were included in the calculation of ACPR28 - why are there 348 patients in this analysis?

d) Figure 7 - it would be helpful to explain that the shaded area on the xy plane shows concentrations required to achieve at least 95% ACPR.

e) Figure 8. Caption is not clear and should be changed to: "Concentrations required for 95% ACPR28: Isobolograms…." Footnote "actual observed individual exposures" - do you mean predicted from PK model? Or measured day 7 concentrations?

f) Line 516. Equation is not readable
g) The effect of K13 mutations was not significant but could you present the effect size, 95% CI and p-values anyway. It is unclear if there was some effect but it was not significant due to small sample size.

e) Why region was not included as the main effect in the model when it's interactions are?

j) Supplementary S4. Table 2 - could you present 95% CI and p-values. Figure 2 - how did you calculate population predicted probability of ACPR28 from the logistic model? - If by simulation method described below the figure - it needs to be stated in the figure caption.

7. Dose-Response simulations

It is unclear how the "exposure-response parameters were sampled from their estimated uncertainty distributions" - can you explain please. Also, the logistic model predicts probability of ACPR28 for each patient - how was this converted into %ACPR28 in Table 7 or probability of ACPR28 in Figure 2 of S4?

8. Discussion

a) a) 622-626 Could you put your findings for parasite clearance in the context of parasite clearance with ACTs and the other artefenomel study (Pyo 2016) in which "artefenomel provided a parasite clearance rate that was slower than that with artesunate on artemisinin-sensitive parasites, but slightly faster than that of artesunate on artemisinin-resistant parasites" (Woodrow, 2017)

b) 658-662. I am a bit confused by the list of study limitations. The study was on malaria patients, the actual drug levels were measured/estimated and the manuscript does not mention weight base dose adjustment.

c) 664-666 this statement is unclear to me. What model are you referring to?

Minor

9. Abstract. Abbreviation without definitions are used ACRPR28, PP, PCT1/2
10. Table 1. Format of the table could be improved to make it more concise, for example Mean and range can be presented as mean(range) on one line; symbol ≥ can be used instead of >=; numbers for females (or males) could be omitted.

**Are the methods appropriate and well described?**
If not, please specify what is required in your comments to the authors.

No

**Does the work include the necessary controls?**
If not, please specify which controls are required in your comments to the authors.

Yes

**Are the conclusions drawn adequately supported by the data shown?**
If not, please explain in your comments to the authors.

Yes

**Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?**
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