Author’s response to reviews

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

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Dear Editor,

We are very grateful for the comments and suggestions of the three reviewers for our manuscript. We are convinced that this has further ameliorated the readability and clarity of the manuscript. Please find attached a point-to-point response to all comments and the modified manuscript with changes highlighted.

Yours sincerely,

Michael Ramharter

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 Fiona Macintyre; Yeka Adoke; Alfred B Tiono; Tran Thanh Duong; Ghyslain Mombo-Ngoma, MD, PhD, MSc; Marielle Bouyou-Akotet, MSc, PhD; Halidou Tinto; Quique Bassat; Saadou Issifou; Marc Adamy; Helen Demarest; Stephan Duparc; Didier Leroy; Bart E Laurijssens; Sophie Biguenet;
Dear Dr Ramharter,

Your manuscript "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" (BMED-D-17-00642) has been assessed by our reviewers. They have raised a number of points which we believe would improve the manuscript and may allow a revised version to be published in BMC Medicine.

EDITORIAL COMMENTS

In addition to addressing the reviewers' comments, we'd also ask that you address the following editorial requests:

1- The 'Ethics approval and consent to participate' statement in the Declarations section should be revised to list the full names of all the ethics committees (institutional and regional) that granted approval for the study. If necessary, the list can be provided as a supplementary file and referenced in the ethics approval statement within the main manuscript

COMMENT: An email has been sent out to the site PIs and the names of the ethics committees have been collected.

CHANGES: A list containing all names of the ethics committees has been added in the supplemental file.
2- Please clarify the role of funders in the 'Funding' statement within the Declarations section

COMMENT: The following statement has been added to the manuscript.

CHANGES: The study was funded by Medicines for Malaria Venture. MMV is funded by a number of donors. Unrestricted funding from a number of donors including; US Aid, Bill and Melinda Gates Foundation, UK Department for International Development, Norwegian Agency for Development Cooperation, Irish Aid, Newcrest Mining Limited, Australian Aid, Swiss Agency for Development and Co-operation and Wellcome Trust, contributed to the study. Study activities at the CERMEL, Gabon were supported financially by the Federal Ministry of Science, Research and Economy of Austria as part of the EDCTP-2 programme. These activities at the Gabonese site are part of the EDCTP2 programme activities of Austria supported by the European Union. These funders had no role in the design, conduct or analysis of the trial.

3- Please clarify the role of Sanofi-Aventis in the 'Acknowledgments' section

COMMENT: This has been specified in the modified manuscript.

CHANGES: We would like to acknowledge our development partner Sanofi-Aventis who are, with MMV responsible for co-development of Artefenomel combinations. Also we would like to thank Elizabeth Cloete, Shobana Gowri Shankar, Illze Crous from Quintiles IMS for their support in the design and analysis of this study.

4- Please include a complete CONSORT checklist (http://www.consort-statement.org/)

COMMENT: We have used the link and filled the checklist accordingly.

CHANGES: CONSORT checklist attached.

The reviewers’ reports, together with any other comments, are below. Please also take a moment to check our website at http://bmed.edmgr.com/l.asp?i=91885&l=P4VG1RWB for any additional comments that were saved as attachments. Please note that as BMC Medicine has a policy of open peer review, you will be able to see the names of the reviewers.
If you are able to fully address these points, we would encourage you to submit a revised manuscript to BMC Medicine. Once you have made the necessary corrections, please submit online at:

http://bmed.edmgr.com/

If you have forgotten your username or password please use the "Send Login Details" link to get your login information. For security reasons, your password will be reset.

A point-by-point response letter must accompany your revised manuscript. This letter must provide a detailed response to each reviewer/editorial point raised, describing exactly what amendments have been made to the manuscript text and where these can be viewed (e.g. Methods section, line 12, page 5). Please also ensure that all changes to the manuscript are indicated in the text by highlighting or using track changes. If you disagree with any comments raised, please provide a detailed rebuttal to help explain and justify your decision.

Please also ensure that your revised manuscript conforms to the journal style, which can be found in the Instructions for Authors on the journal homepage.

A decision will be made once we have received your revised manuscript, which we expect by 13 Jul 2017.

Please note that you will not be able to add, remove, or change the order of authors once the editor has accepted your manuscript for publication. Any proposed changes to the authorship must be requested during peer-review, and adhere to our criteria for authorship as outlined in BioMed Central’s policies. To request a change in authorship, please download the 'Request for change in authorship form' which can be found here - http://www.biomedcentral.com/about/editorialpolicies#authorship. Please note that incomplete forms will be rejected. Your request will be taken into consideration by the editor, and you will be advised whether any changes will be permitted. Please be aware that we may investigate, or ask your institute to investigate, any unauthorized attempts to change authorship or discrepancies in authorship between the submitted and revised versions of your manuscript.
I look forward to receiving your revised manuscript and please do not hesitate to contact us if you have any questions.

Best wishes,

Anita Marinelli, PhD
BMC Medicine
https://bmcmedicine.biomedcentral.com/

Reviewer reports:

Reviewer #1 Kasia Stepniewska:
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.

COMMENT: We agree with the reviewer and made the respective changes.

CHANGES: Definition of Endpoints was inserted before statistical consideration.
"An additional key exploratory objective was to characterise the dose / exposure response relationship for the combination for the primary efficacy end point across the patient population,
and to identify significant covariates influencing efficacy. Safety, tolerability and pharmacokinetics (PK) was also assessed. Details of the study objectives, design and end points are summarised in S1 Study Protocol Section 1 Study Synopsis and in more detail in Sections 4, 5.1 and 5.10 respectively."

A section on parasite counting was added.

"A slide was considered negative in the absence of asexual parasites per 1000 counted leukocytes using a 100X magnification oil immersion objective. Parasite density was calculated as follows: (number of counted parasites / counted leukocytes) x most recent absolute leukocyte count per microliter."

PCR genotyping:

COMMENT: We agree that providing information about PCR genotyping would increase the value of the manuscript.

CHANGES: Passage added on PCR genotyping in the 'Endpoints' section.

"Three polymorphic genetic markers MSP1, MSP2, and GluRP were used to distinguish recrudescence from new infections, according to WHO recommended procedures and as previously described by Snounou et al.. Recrudescence was defined as at least one identical allele for each of the three markers in the pretreatment and posttreatment samples. New infections were diagnosed when all alleles for at least one of the markers differed between the two samples."

Pharmacological sampling: Information needed

COMMENT: We agree with the reviewer and made the respective changes in the paragraph 'Pharmacokinetic Analysis'.

CHANGES: "In adult patients (>35kg) blood samples for pharmacological analysis were collected at 15 to 16 time points: pre-dose, 2, 4, 6, 12, 24, 48, 72 hours and days 5, 7, 10, 14, 21, 28, 42, 63. In paediatric patients the number was between 3 and 10 samples."
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.

COMMENT: We agree with the reviewer and have made the respective changes.

CHANGES: "For the PP population, the number of recrudescences and new infections with 95% CI for ACPR from day 28 to day 63 post dose has been added to Table 4. In addition, the number of recrudescences and new infections with 95% CI for ACPR from day 14 to day 63 post dose by region has been added to the text (lines 389-399). Note up to Day 7 failures are assumed to be failure to clear the initial infection. Also weekly meetings occurred only up to day 28. Regarding the Kaplan-Meier analysis, please see the response below."

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

COMMENT: The numbers at the top of the whiskers in Figure 2 are the percentage ACPR28.
CHANGES: A statement to this effect has been added to table 2 (Table footnote).

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

COMMENT: We have provided KM with a y axis scale range of 0.5 to 1, although we have reservation about this because we believe that this over-accentuates the rate of recrudescence. We have also provided the Kaplan-Meier plots as survival free risk as suggested by the other reviewer.

Given that the Kaplan-Meier evaluates data for each patient over the study period, and that the analysis is carried out per dose, region and age category, we do not recommend including the numbers at risk in the manuscript because of the large number of data points. The tables attached provide summary information over the study period of events and censored patients for your information. In addition the relevant SAS output from these procedures with the calculated
probabilities as presented in the Figures, is provided in EXCEL format for reference. Generally the model procedure steps in SAS would not be presented in a table only the actual outcome.

In addition, we would like to clarify that the duration per patient is based on the data handling conventions as expected for a Kaplan-Meier analysis.

Censored: Patients with no event are censored at the time of study completion, premature study discontinuation, including switch to established anti-malarial treatment, or start of any other treatment with anti-malarial activity as captured on the Prior and Concomitant Medications eCRF, whichever is earliest. As is standard practice for a Kaplan-Meier, those patients with an event of recrudescence or new infection have a contribution to the Kaplan-Meier based on the derived duration until the start of the event. For those patients with no event, their contribution to the Kaplan-Meier is based on the actual study duration with the cut-off set appropriately for the given study design and indication. In this way the curves are accurately calculated based on the SAS procedure stipulated above.

As this is a manuscript, adding the lengthy probability tables to the manuscript would not be a feasible option in view of three treatment arms over a potential 63-day study period in addition to region and age group classification.

CHANGES: Modified figure and KM plot added to the manuscript

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?)

COMMENT: We agree with the reviewer and have modified the respective paragraphs.

CHANGES: For ACPR, greater detail has been provided for the PP population up to Day 14, 21, 28, 42 and Day 63, including 95% confidence intervals for recrudescence and new infection.
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?

COMMENT: Yes, this is the proportion of patients who cleared parasites by 72h post-dose, expressed as a percentage.

CHANGES: The word median has been removed as it was incorrect.

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."

COMMENT: This statement is simply based on looking at results across piperazine dose. E.g. for the African population the % patients with parasites cleared by 72h post dose was 92.9 (84.4, 96.9)%, 96.5 (91.3, 99.0)% and 89.3 (82.0, 94.3)% for 640, 960 and 1440mg piperazine respectively. The use of statements like 'no clinically significant differences' was avoided as this was not tested.

CHANGES: The wording has been changed in the text.

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.
COMMENT: PCt1/2 was estimated using the WWARN Parasite Clearance Estimator, based on the linear part of the individual natural log parasitaemia-time profiles. Clearance rate constant (1/hours) was defined as the minus slope of the final model fitted after exclusion of outliers, lag phase and tail. Parasite clearance half-life (hours) is the estimated time for parasitaemia to decrease by half. Data from patients with a poor fit to the linear model ($r^2 < 0.75$) or with < 3 data points are excluded from the analysis. This has been added to the text. The interquartile range has been replaced in the text with minimum and maximum (i.e. range) as requested.

Regarding subgroups, as mentioned in the succeeding paragraph, region (due to the high level of Kelch mutation in Asia) was the key factor affecting PCt1/2. There was no clear difference between African patients >5 years and <5 years or any other population.

CHANGES: "Initial clearance of parasites was rapid in the African population. Median parasite clearance half-life (PCt1/2) was calculated using the WWARN Parasite Clearance Estimator (PCE) details of which are published.[26] PCt1/2 is the estimated time for parasitaemia to decrease by half, derived from the clearance rate constant 1/hours. Parasite clearance values were reported only for results with $R^2 > 0.75$. PCt1/2 was longer in Vietnam versus Africa (6.1 hours [minimum 1.14, maximum 12.73] versus 3.5 hours [minimum 1.24, maximum 7.71]). Within Vietnam, PCt1/2 was similar across the four study centres."

It is unclear what "within Vietnam, PCT1/2 was similar across study centre" means.

COMMENT: there were 4 study centres in Vietnam and the PCt1/2 values were similar in all of them.

CHANGES: The wording has been changed slightly.

e) All K13 mutations detected should be listed.

COMMENT: We agree that this would be beneficial for the value of the manuscript and therefore we added all the K13 mutations to the text.
CHANGES: All kelch mutations have been added and current Figure 5 (previously Figure 4).

"A total of 20 known Kelch13 genotypes were tested for (in Africa 4 new genotypes were identified at low frequency (0.3-1.7%); A578S, A626V, M562T, Y541F, none associated with artemisinin resistance)[27]. In Vietnam, a high frequency of Kelch13 mutation was observed (70.1%). Five mutations were detected, four of which, C580Y, I543T, P553L and V568G are defined according to WHO[27] as validated or candidate markers for partial artemisinin resistance, the exception being C469P which is not known to be associated with artemisinin resistance[27]. "

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.

COMMENT: Parasite clearance values were reported only for results with R2>0.75.

CHANGES: All kelch mutations have been added and current Figure 5 (previously Figure 4).

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?

COMMENT: Whilst there are PCt1/2 values > 5 hours in Africa, the majority of PCt1/2 values for WT in Africa are < 5 hours. This is not dissimilar to the scatter of PCt1/2 values indicated in the African population in the publication by the WWARN Parasite Clearance Study Group (Malaria J 2015) which you mention (Figure 5 showing PCt1/2 by age). Note the majority of African patients in our study were < 5 years of age. We therefore consider that the data is not at odds with the WWARN publication.

Theoretically the RATE of parasite clearance (i.e. the log-linear decline) of a single drug is specific to that drug (its mechanism of action) and the parasite population (which could differ between region / sub-region even in the absence of resistance). In addition, there may be potential difference in sampling time etc between studies. Given these considerations, we consider that the only way to test for a difference in PCt1/2 between drugs is a side by side
comparison within one study. Hence we prefer not to refer to other publications. We have not made any changes to the text.

As before, parasite clearance values were reported only for results with R2>0.75.

4. Safety and Tolerability.

a) Timing of TEAE/TESAE should be included where relevant.

COMMENT: We agree with the reviewer.

CHANGES: Timings were added.

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.

COMMENTS: We agree with the reviewer.

CHANGES: Table 5 was removed.

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)

COMMENT: You are correct. For all patients day 7 blood samples, to be analysed for plasma concentrations of both piperaquine and artefenomel, were scheduled. However, not all patients provided day 7 plasma concentrations (no sample, not analysable, below limit of quantification). Moreover, there is variability in the actual time the sample was taken, even if the impact of this is probably low. For example, only 288 patients out of 348 (current exposure-response analysis) had measurable day 7 (between 144 and 192 hrs post dose) artefenomel concentrations.

We are confident that the model-predicted day 7 concentrations are reasonable estimates of the concentration at day 7. The population popPK models for both artefenomel and piperaquine described the observed Cday 7 concentrations well (figures 2 to 7 in S3). In addition, the individual observed PK profiles (incl around day 7) were very well described by the individual model fits (not shown because of restrictions in space, but an example was given in figure 1 S4).

Figures 4 and 7 were added to S3, showing the observed vs individual predicted concentrations around day 7 for artefenomel and piperaquine respectively.

We did not do an exposure – response analysis using the observed concentrations. We estimated the day 7 concentrations (168 hrs post dose) for all patients using all information available (population PK analysis). These model estimated values were used for our PK summaries as well as for the exposure-response analysis. This is common practice in the pharmacometric community, allowing the use of concentration estimates at precise (not observed) timepoints, account for residual variability, and address missingness (in this case not at random: low (BQL) exposures are associated with treatment failure). We do not believe that an exposure-response analysis on the observed values only would contribute to our understanding or address any specific concerns.
CHANGES: To address the potential confusion between observed and model-estimated concentrations at day 7, we added “estimated”, before Cday at key places in the text. The method section (Pharmacokinetic Analysis) already contains the phrase: “Subsequently, exposures of artefenomel and piperaquine for each patient were derived from the individual PK parameters estimated in the population PK analysis. Cmax, Tmax and concentration on day 7 (Cday7) were obtained from the simulated profiles, and AUC extrapolated to infinity (AUCinf) was calculated directly from the estimated PK parameters”, with more detail in S3 Pharmacokinetic Analysis Details.

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 recrudescences which is relevant when exploring effect of the concentration at day 7. This analysis may give you more power to explore effects of covariates.

COMMENT: No, we did not consider a survival regression model. This may indeed have given us more power. Thank you for the suggestion. We may consider this in the future. Our interest was in describing the exposure-, and subsequently dose-, relationship to the PCR-adjusted ACPR on day 28 specifically. The PCR-adjusted ACPR28 was our main efficacy criteria for clinical success.

NO CHANGES MADE.

c) 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.

COMMENT: We agree with the reviewers.

CHANGES: The caption for Figure 6 (now Figure 7) now reads: “Summary of the observed ACPR28 by estimated artefenomel concentration at day 7 together with the logistic regression model predictions”
COMMENT: Originally the bin sizes were uneven (but reflected in the confidence intervals) because the binning was performed before stratification.

CHANGES: We have re-issued the plot (Fig06_REV), now stratifying before binning. We added information regarding the binsize to the legend of the figure: “Number of Patients per bin: Africa ≤ 5y n=53, Africa > 5y n=15, Asia n=20”

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

COMMENT: The numbers are different because the table and the exposure-response used different populations. Table 4 refers to the PP population which excludes patients considered to have major protocol violations, which included patients with parasitemia levels significantly below and significantly above the protocol inclusion criteria. However, these patients were considered informative to the exposure-response analysis and therefore were included.

For the exposure-response analysis, therefore, “all patients in the ITT set with ACPR28 values and exposure for both drugs were included in the analysis” (line 294 manuscript). The difference with the PP population was mostly patients with parasitemia levels outside the inclusion criteria and subjects with administration irregularities. Both types of subject are particularly informative for exposure – response.

NO CHANGES MADE.

e) 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.

COMMENT: We agree with the reviewer.

CHANGES: We added the following sentence to the legend of the figure: “.The shaded area shows the concentrations required to achieve a probability of ACPR28 greater than 0.95.” (now Figure 8)
f) 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?

COMMENT: We agree with the reviewer that this was not clear enough.

CHANGES: Caption and legend have been changed: “Figure 8. Concentrations associated with a probability of ACPR28 of 0.95: Model predicted Isobolograms by Region and Baseline Parasitemia

Asian Patients = Red: African Patients= Blue: Baseline parasitaemia 10,000 par/µL: Solid isobole; Baseline parasitaemia 100,000 par/µL: dotted isobole; . Includes actual estimated individual exposures associated with Cure (ACPR28): open symbol or Failure (recrudescence): closed symbol”

g) Line 516. Equation is not readable

COMMENT: We agree.

CHANGES: The equation has been re-inserted as word text

\[
\log\left(\frac{p}{1-p}\right) = 3.23 + 0.22 \times \text{Cday7PQ} + (0.73 - 0.59[\text{if Asia}]) \times \text{Cday7OZ} - 1.27 \times \log_{10}(\text{BasePar}) + 0.46 [\text{if Asia}]
\]

h) 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.
COMMENT: This is indeed relevant. There is already a sentence in the discussion (“Caution should be exercised here as the sample-size may well not have been sufficient to identify any association between Kelch13 and ACPR28 in the exposure – response analysis.”) highlighting this issue.

CHANGES: We added a table and a paragraph to S4:

„No significant effect of the factor Kelch13 genotype was identified. The results are summarized in table 3. The results suggest that there may be some effect (WT having a higher probability of ACPR28), but that the sample size was too small to identify it.

Table 3: Summary of the evaluation of Kelch13 genotype added to a base model with artefenomel and piperaquine Cday7 included (complete case only analysis, n=297).

<table>
<thead>
<tr>
<th>Factor</th>
<th>Estimate (WT)</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>True Wild Type (WT; no mutation) vs. any mutation (MUT)</td>
<td>0.78 (-0.08 – 1.66)</td>
<td>0.0760</td>
<td></td>
</tr>
<tr>
<td>C580Y and I543T mutations (MUT) vs. WT for these 2 alleles</td>
<td>0.89 (-0.25 – 2.08)</td>
<td>0.1298</td>
<td></td>
</tr>
<tr>
<td>C580Y, I543T as well as P553L and V568G (MUT) vs. WT for these 4 alleles</td>
<td>0.88 (-0.07 – 1.86)</td>
<td>0.0720</td>
<td></td>
</tr>
</tbody>
</table>

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

COMMENT: Not sure whether we understand the question correctly. Region was included as a main effect in the model (the additional intercept for Asian Patients): See equation and table 2 of S4 Exposure response analysis details.

j) Supplementary S4. Table 2 - could you present 95% CI and p-values.
COMMENT AND CHANGES: Table 2 in S4 has been updated with the requested information

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.

COMMENT: The method was similar to the one described in the simulation method, but a replicate was based on the exact study population (including the estimated individual Cday7 and other covariates, as well as the stratification by TMT and region/age). The main difference with the simulation method described under the figure for the dose – response is that for the diagnostic figure 2 we did not simulate the PK (based on the covariates) but used their individual estimated exposures and used the exact study population. Therefore, the diagnostic plot reflects the performance of the exposure – response analysis only and not the popPK as well.

CHANGES: We added the following sentence to the caption of figure 2 in S4

“The simulations were similar to the simulation method described below, but based on the actual patient population in each subgroup (i.e. estimated individual Cday7 and covariates in those patients) and across 1000 replicates.”

7. Dose-Response simulations

It is unclear how the "exposure-response parameters were sampled from their estimated uncertainty distributions” - can you explain please.

COMMENT: Each simulated replicate used a set of model parameters drawn from the multivariate normal distribution based on the estimated variance-covariance matrix (separately for each popPK and exposure-response model).

CHANGES: This sentence was added to the simulation method section.
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?

COMMENT: For each patient (out of 2000) within a replicate the probability of ACPR28 was simulated, followed by treatment outcome (cure or failure, one draw based on that probability). These TMT outcomes were then summarised across the 2000 patients to generate a %ACPR (successes/2000).

For figure 2 S4, the procedure is similar, but here the PK was not simulated (actual estimated individual exposures were used) and the exact study population was used as described in the response to comment 6j.

CHANGES:

The simulation method (S4) now reads:

“Two thousand patients per simulated treatment arm were sampled from the relevant study population, while maintaining the covariate structure within each sampled patient (e.g. body weight, age, baseline parasitemia). A set of population PK parameters and exposure-response parameters was sampled from their estimated uncertainty distributions. That is, each simulated replicate used a set of model parameters drawn from the multivariate normal distribution based on the estimated variance-covariance matrix (separately for each popPK and exposure-response model). Then, for each patient, a Cday7 for artefenomel and piperaquine and subsequently, a probability of ACPR28 followed by treatment outcome (success or failure) was simulated, using the developed models, the set of sampled parameters, each patient’s covariates, and the artefenomel and PQP doses. The treatment outcomes for the 2000 patients were then summarised by treatment (number of successes/2000) to generate an estimate of %ACPR28. These steps were repeated 750 times (replicates), each time sampling a new set of patients and parameters. For each simulated treatment arm the ACPR28 across replicates was summarised to generate a point estimate and 90% Confidence Interval (median, 5th and 95th percentile of the distribution across replicates).”
8. Discussion

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)

COMMENT: As mentioned in the response to a previous point, the rate of parasite clearance is related to the specific mechanism of the drug and the parasite population, which may differ by region / sub-region. Therefore although artefenomel is likely to have a similar mechanism of action to artesunates, there may be some differences and hence a different (slower) PCt1/2 is possible. However, we believe the PCT1/2 can only be compared within study.

With respect to the finding in the Phylo publication (also referred to in Woodrow 2017), these data were obtained on the Thai- Cambodian border and only a small number of genotyped samples with PCt1/2 values were reported. The median PCt1/2 of wild-type (n= 11), was 4.42h, minimum 1.99h, maximum 8.7h. Only one of the kelch 13 mutations (C580Y, n=4, PCt1/2: 7.33, 9.2, 4.1 and 5.59h) was common to both studies. Several of the other mutations have not been confirmed as related to artemisinin resistance. The median PCt1/2 value for all mutations was 5.50, (minimum 2.86, maximum 7.61). Given the small amount of data and the difference in region and kelch13 mutations detected, we do not think this is a suitable publication to refer to within this publication.

We are currently genotyping additional samples obtained from the study both for kelch13 mutations and for piperaquine resistance markers, and we will examine the relationship between these genotypes and both PCt1/2 and ACPR28. Within this publication we will discuss the results in the context of the wider findings on resistance.

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.

COMMENT: Exposure in young children was lower than older subjects, however since there was a significant proportion of the population who vomited, and anecdotal reports that the young
children were unable to consume the entire dose, it could not be assumed that the intended dose had been truly administered. Hence it was not possible to conclude whether the low exposure was due to over estimation of clearance (and hence underestimation of the dose required to achieve the same exposure as adults), or whether the younger patients were not effectively dosed.

We have tried to make this clearer.

Dose adjustment is mentioned in the study design section, and we added some additional detail.

CHANGES:

In the study design section:

Fasted patients >35kg received artefenomel 800mg in loose combination with PQP doses of 640, 960 or 1440 mg at day 0. Patients <35 kg received body weight-adjusted doses within weight bands predicted to achieve similar exposure ranges to patients >35 kg. The dose for a given weight band was adjusted by scaling clearance allometrically, using the relationship CL = (body weight/70)0.75).

In the discussion section:

A significant limitation of the study was that although the formulation used had been tested in adult healthy subjects, it had not been tested in adult, and more importantly paediatric malaria patients. It is possible that the palatability of the formulation and/or volume of administration contributed to the higher than expected rate of vomiting in the study. In addition, although compliance data on drug consumption was collected, insufficient detail was recorded to truly capture compliance since despite a high reported success rate of drug administration, there were anecdotal reports that young children were unable to ingest the full dose. The effect of this was that the study was unable to conclude on the best weight based dose adjustment for patients <35kg i.e. drug exposure in young children was lower than in adults, however it could not be
concluded whether this was due to the weight based dose adjustments or because the intended dosage was not successfully administered.

However, the dose – response simulations suggest that even if the young children had similar exposures to the adults as well as no vomiting, the target efficacy would not have been achieved.

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

COMMENT AND CHANGES: We agree with the reviewer, thus we removed the statement.

Minor

9. Abstract. Abbreviation without definitions are used ACRPR28, PP, PCT1/2

COMMENT AND CHANGED: Definitions were added.

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.

COMMENT: We agree with the reviewers and made the suggested changes.

CHANGES: Symbols were changed as suggested. MINIMUM and MAXIMUM were truncated as MIN and MAX. Numbers and percentages for females were deleted. And inadvertent error was noticed and corrected (numbers and percentages in the 'Africa-Part' of the table had been filled into wrong columns. Now numbers add up correctly to the totals in the top of the table).
Reviewer #2 Cor Jesus Fontes:

- Macintyre F and colleagues performed 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. The manuscript is quite extensive, but very well written, well analyzed and has its results clearly shown and well discussed.

- The primary objective of the study was to determine whether a single dose combination of 129 artefenomel plus piperaquine is an efficacious treatment for uncomplicated P. falciparum malaria. Secondary and exploratory objectives included determination of the incidence of recurrence, recrudescence and new infection, estimation of parasite clearance kinetics and exploration of the relationship between Kelch13 genotype and parasite clearance half-life (PCt1/2) in Asian patients. An additional key exploratory objective was to characterise the dose/exposure response relationship for the combination for the primary efficacy end point across the patient population. Safety, tolerability and pharmacokinetics (PK) was also assessed in the study.

- Since the study proposes the introduction of a single dose antimalarial regimen, the following points highlight its relevance: poor adherence to the standard malaria treatment in endemic areas impacts malaria morbidity and mortality and contributes to development of parasite resistance; An effective cure obtained by a such single treatment, directly observed if required, would also provide an important tool to support malaria elimination efforts.

- Artefenomel 800mg was administered in loose combination with three doses of PQP (640, 960,1440 mg) to 3 groups of patients. Although none of the treatment arms reached the target efficacy of ≥95% PCR-adjusted ACPR (adequate clinical and parasitological response) at Day 28, the results found in this phase II trial are very promising in the current context of malaria therapy.

- The manuscript is suitable for publication, without revision.

COMMENT: We are grateful for the reviewer’s appreciation of our work.

CHANGES: none
Reviewer #3 Sam Salman:

A negative trial which demonstrates that a single dose ACT combination of artefenomel and piperaquine is not likely to be suitable at any tolerable/safe dose.

Some concerns with regards to the presentation of data, analysis of exposure/response and PK analysis need clarification or correction.

Table 2: The percentages are not consistent with the totals and number - for example n=437 in the total column on the right, in the first row 437 is expressed at 97.5%. It appears the percentage refers to the total randomised patients (437/448) rather than the safety set which the table refers to.

COMMENT: Yes, we agree with the reviewer.

CHANGES: Table 2 had incorrect header information which we have rectified. We have also clarified the populations in the text.

Lines 340-345: The final comment in this section "... despite reported compliance being >95% in all populations." is discordant with the provided information on compliance 65% in the African and 91.5% in the Asian population.

COMMENT: We agree with the reviewer that the distinction was not clear enough.

CHANGES: The wording has been changed to make clearer distinction between compliance and full drug consumption

Table 4: When comparing this table to Table 3 it is not clear why the denominator changes from the crude ACPR to the PCR-adjusted ACPR. For example day 42 in table 3 for 800:1440 dose has a crude result of 68/146 and PCR adjusted result of 73/146 while in table 4 it is 67/130 and 72/100 respectively. Although it is understandable that there is a smaller number in the PP
analysis set is it not clear why there should be 30 less participants in the PCR adjusted ACPR in this same set. The same pattern exists throughout this table.

COMMENT: The definition of crude and PCR-adjusted ACPR for the ITT and PP populations are according to the WHO 2009 guidance METHODS FOR SURVEILLANCE OF ANTIMALARIAL DRUG EFFICACY (Appendix 9), and is detailed in the SAP (Section 15.3). There are 3 outcomes, cure, failure or missing and patients’ treatment response is classified differently for ITT and PP and for crude and PCR-adjusted outcomes.

This results in different denominators depending on the analysis population and whether crude or adjusted proportion is being derived. For the ITT population, only cure or failure outcomes are allowed (so missing response results are classified as failure) and so the denominator for ITT does not change over time (except for Day 63, where only patients who agreed to extend the study were included). In addition, only one difference in the handling of treatment outcome exists between the crude and PCR adjusted ACPR and hence the denominators are very similar (in the case of our data (Table 3), there was no difference in denominator.

For the PP analysis, in certain circumstances the result is set to missing and hence the denominator tends to decrease over time as more subjects' outcomes are set to missing as these are not included in the analysis. There are also two circumstances in which the treatment outcome for crude and PCR-adjusted differ and hence the denominator differs between crude and PCR adjusted.

CHANGES: Text has been added to lines 244-247 to clarify the documents to refer to, and the footnote to Table 4 has been expanded.

Figure 3: Consider presenting the plots as survival free from recrudescence.

Lines 412-413: It would be of benefit to present data to support the comment that "No association between Kelch13 mutation and ACPR28 was identified." This could be included in the text.
COMMENT: Regarding the comment about the relationship with ACPR, as per the other reviewers comment we have removed the statement about the relationship between Kelch13 and ACPR. Instead we simply discuss this relationship through the modelling.

CHANGES:

The KM plots have been changed to survival free.

Table 6 (now Table 5): The reported percentage of Malaria + Pf infection (27.9+5.9=33.8%) is lower than suggested from the efficacy data. For example, crude ACPR 42 days reported as 45.1% (197/437) therefore 54.9% (240) had malaria diagnosis at this time. The reported rate of vomiting here (11.4%) differs from that in the text (line 342 - 28.8%).

COMMENT: The % incidence of malaria reported as a treatment-emergent AE up to day 28 was 27.9% which is less than the true incidence because not all incidences of malaria infection were reported by investigators as AEs. Note also that the malaria incidence and the Pf incidence are not necessarily additive, because investigators may report both AEs in a single patient.

CHANGES: We have corrected the title of Tables 6 (now Table 5) since treatment-emergent AEs were calculated only up to Day 28.

Lines 481-482: There is a very high CV in the AUC and Cday7 values (both >100%) the impact of high CV on target concentration attainment, particularly with a single dose therapy, should be discussed.

COMMENT:

The study results also illustrate the challenges in developing a single encounter combination treatments. Firstly, the administered dose needs to be higher to achieve the required duration of exposure compared to multiple day treatments, therefore the ratio of Cmax to overall exposure will be greater. And secondly, high between subject variability in drug exposures, due in part to a limited number of bodyweight bands for dosing (and which in this study may have been compounded by challenges in administering large dosing volumes to sick children), in addition to a large between subject variability in baseline parasitaemia and potentially parasite sensitivity,
means that the majority of patients will be required to be 'overdosed' if a very high cure rate is to be achieved with a single (adult equivalent) dose level. Both factors mean that a wide therapeutic window is required.

CHANGES: A paragraph addressing this was added to the discussion:

"The study results also illustrate the challenges in developing a single encounter combination treatments. Firstly, the administered dose needs to be higher to achieve the required duration of exposure compared to multiple day treatments, therefore the ratio of Cmax to overall exposure will be greater, and secondly, high between subject variability in drug exposures, due in part to a limited number of bodyweight bands for dosing (and which in this study may have been compounded by challenges in administering large dosing volumes to sick children), in addition to a large between subject variability in baseline parasitaemia and potentially parasite sensitivity, means that the majority of patients will be required to be 'overdosed' if a very high cure rate is to be achieved with a single (adult equivalent) dose level. Both factors mean that a wide therapeutic window is required."

Lines 513-514: It is not clear if the interaction between region and artefenomel Cday7 was included before or after the assessment of the effect of Kelch13 mutation. Given that the mutation was only present parasites from Asian participants and a higher day 7 concentration was required to achieve the same efficacy in these participants, it would be important to know that the potential interaction of these mutations was investigated completely before incorporating another interaction.

COMMENT: The effect of the Kelch13 mutation was evaluated on a base model including only artefenomel and piperaquine Cday7. All covariates were evaluated separately in the base model. And whereas region was significant (p=0.0044), none of the various Kelch13 groupings were.

In addition the paragraph in S4 describing the covariate selection was modified to make the procedure followed clearer:

CHANGES: More detail regarding the effect of the Kelch13 mutation was added to S4, including an extra table.
“First, a base model that included the effect of artefenomel and piperaquine Cday7 was developed. Univariate effects of either artefenomel or piperaquine day7 were evaluated, relative to an intercept only model. Subsequently, the univariate effects of the other covariates were evaluated (in the presence of both artefenomel and piperaquine Cday7). The significant covariates were retained in the model after which interactions were explored”

Line 516: The equation was distorted in the pdf for review.
COMMENT: We thank the reviewer for pointing this out.

CHANGES: This has been replaced in the text

\[ \log_{10} \left( \frac{p}{1-p} \right) = 3.23 + 0.22 \times \text{Cday7PQ} + (0.73 - 0.59 [\text{if Asia}]) \times \text{Cday7OZ} - 1.27 \times \log_{10}(\text{BasePar}) + 0.46 [\text{if Asia}] \]

Table 7: This simulation exercise predict a ACPR of 92% (89-95%) for the highest dose in this study, much higher than the observed 84 and 62% in those 2-5 and <2 years, respectively. It may be of greater value to perform simulations with results closer to the observed results. It would be of interest to model 2 or 3 dose regimens.

COMMENT: The objective of the dose response simulation was to evaluate what dose combinations might meet our target efficacy in our target population, assuming that we manage the observed administration issues (vomiting, compliance). Therefore, the simulated dose – response are not comparable to the study results.

The performance of the exposure – response model was evaluated as well (through simulation and comparing to observed results) and shown in Figure 2 of S4.

Indeed, simulations 2 and 3 day dosing regimens are of interest and were explored, but it was decided not to include these in the publication since the focus of the study and paper is on single encounter treatment. In addition, the publication is already quite lengthy.
Line 652: The tolerability is noted as "generally good", it is difficult to marry this comment with the observed rate of vomiting of 28.8%.

COMMENT: We agree with the reviewer.

CHANGES: We have removed this statement.

PK analysis: A non-linear PK relationship was identified for artefenomel. It would be more common for the nonlinearity to be related to concentration (ie michaelis menten elimination) rather than dose. A justification for using dose to model nonlinear elimination should be provided.

COMMENT: We agree with the reviewer. Modelling the non-linear PK as a function of concentration is the preferred approach. Modeling as a function of dose was a considered an (adequate) approximation, mainly in the interest of time. We do believe that it was adequate for the main objectives of the popPK analyses: estimating the individual exposures, and subsequent exposure – response analysis. The individual profiles were well described.

It may have an impact on any dose response simulations, in particular when extrapolating. But in reality we are limited to the 800 mg dose, or very close (lower does not make sense since we would like artefenomel to perform as good as possible, and 800 mg would already be sub-optimal), and we believe the model performs adequately at that dose level (see VPCs in S3).

In conclusion, we agree that MM elimination should be explored and would make more sense, but believe that the current model was fit for purpose.

CHANGES:

A paragraph was added to S3 to discuss and explain this:
“The non-linear PK was modeled as a function of dose, rather than concentration (eg Michaelis – Menten elimination) which would be preferred. This approximation was considered adequate, however, considering the main objectives of the popPK analysis: to estimate the individual exposures and to use these to evaluate the exposure – response relationship. For simulations, especially extrapolations, this approximation may be a limitation of the current model. However, the model performed adequately for the dose range included in the analysis (100 – 1200 mg), including the main dose level of interest for single encounter treatment, 800 mg.”