Author's response to reviews

Title: Peripheral blood monocyte-to-lymphocyte ratio at study enrolment predicts efficacy of the RTS,S malaria vaccine: analysis of pooled phase 2 clinical trial data

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Author's response to reviews: see over
Dear Dr Denyer,

We thank the reviewers for their helpful comments on our manuscript and have submitted a revised version with changes as suggested by referee 2 below.

**Major Compulsory Revisions**

1. There are a number of limitations to the study, as acknowledged by the authors, but the most significant is the availability of baseline monocyte and lymphocyte numbers in only two of the 11 Phase 2 studies, and then only at some sites within those two studies. However, according to the original report of the Phase 2 study in Kenya and Tanzania (ref 11; Bejon et al. N Eng J Med. 359:24) authored by the senior author of the current manuscript, a full blood count was done for all participating children at the time of first vaccination. Why, therefore, was the Tanzanian cohort not included in the current analysis?

   For the ML ratio calculation we required both monocyte and lymphocyte counts. Whilst all sites collected lymphocyte counts, only Kilifi, Kenya and Lambarene, Gabon acquired both monocyte counts as a distinct cell population. We have now included the clarified statements below under “Study setting and participants”.

   “Full blood counts, including absolute lymphocyte count, were available for all sites. However, most sites did not collect absolute monocyte counts. Instead, they used cell counters that returned lymphocyte count, neutrophil count and a mixed cell count composed of the sum of monocytes, basophils and eosinophils. Our analysis was therefore restricted to Kilifi, Kenya [1] and Lambarene, Gabon [2] where absolute peripheral blood lymphocyte and monocyte counts were collected as distinct cell populations.”

2. The two cohorts included in this analysis were Kenyan children aged 5-17 months at first vaccination (0,1,2 schedule) and Gabonese infants aged 6-10 weeks at first vaccination (0,1,2 or 1,2,7 schedule). RTS,S efficacy is known to vary between individuals according age at vaccination, as noted by the authors, and the numbers of lymphocytes and monocytes could also be expected to vary with age. Also, the definition of clinical malaria differed between the two study sites (>250 parasites/ul versus > 500 parasites/ul). The manuscript must present separate analyses for each of the two sites, as well as the combined analysis. Particularly since the authors note (page 7) that “ML ratio was significantly correlated with age at vaccination.”
“A tendency towards an interaction between ML ratio and RTS,S vaccination was observed when the cohorts were analyzed separately but did not reach statistical significance (p=0.08 for Kenya and p=0.05 for Gabon).”

3. Why did the authors choose not to identify the association in one cohort and confirm the finding in the second independent cohort?

Our approach was to analyze data from all trial sites together, as this would provide the greatest power to conclusively determine the relationship between ML ratio and vaccine efficacy while accounting for demographic differences between study populations. However, we do agree with the reviewer that testing the association in one cohort and replicating it in another is a strong analytical approach, but we think this would only be possible in larger suitably powered cohorts (such as those in the currently ongoing phase III trials).

4. The authors should speculate as to a potential mechanistic basis of the observed relationship between ML ratio and RTS,S vaccine efficacy. They should also note whether similar outcomes have been observed for other vaccines.

We have now added a few sentences in the last paragraph of the “Results and discussion” section to address this:

“However, recent studies on mouse models have demonstrated suppression of vaccine immunity by inflammatory monocytes and the enhancement of vaccine efficacy against tumours following monocyte depletion at the time of vaccination [3]. Further, inflammatory monocytes have been shown to accumulate and suppress anti-viral T cell responses during chronic lymphocytic choriomeningitis infection in mice [4]. It is plausible that RTS,S vaccine efficacy is specifically inhibited by inflammatory monocytes, thus confounding induction of an effective adaptive response, but further studies in both animal models and humans will be needed to confirm this.”

Minor Essential Revisions

1. Please clarify the time interval between screening (when the full blood count was done) and the time of vaccination. Was this consistent between study sites and would it be expected to reflect the blood count (and hence M:L ratio) at the time of vaccination?
We have added the below statement under “Study setting and participants”:

“The median time interval between measurement of ML ratio at screening and vaccination was 57 days for Kenya and 60 days for Gabon, but this was not expected to confound observed associations since we have previously shown that ML ratios among healthy children are stable over time [5].”

2. The statement that “ML ratio did not directly influence clinical malaria risk in the RTS,S group … but there was a strongly significant interaction between ML ratio and vaccine efficacy” is confusing as written. Please rewrite for clarity.

Done. See paragraph two under “Results and discussion”:

“ML ratio did not directly correlate with clinical malaria risk among individuals in the RTS,S group (HR=1.2, 95% CI 0.58 to 2.66, p=0.6) or among controls (HR=0.7, 95% CI 0.28 to 2.02, p=0.6). However, there was strong evidence for a statistical interaction between ML ratio and vaccine efficacy (p=0.006) suggesting that the protective effect of vaccination is significantly modified by ML ratio.”

3. References – correct the errors in capitalization of journal names.

Done.

Discretionary Revisions
1. The authors should consider presenting the data showing that ML ratio correlated positively with peak anti-CS protein IgG antibody response, since this would be of interest to the readers.

We have now added this as Additional file 1.

On examining the plot it drew our attention to a likely error in calculation that we have corrected. Thus, there was no evidence for an association between anti-CS protein IgG response and ML ratio in either the pooled dataset (rho=-0.06, p=0.3) or in any of the two trial sites (Kenya rho=0.001, p=0.99; Gabon rho=0.08, p=0.4). We have included two statements in the “Results and discussion” section:

“ML ratio showed no association with the peak RTS,S-induced IgG antibody response to the CS protein (rho=-0.06, p=0.3; Additional file 1), but the interaction between RTS,S and
ML ratio was still evident after adjustment for this variable (p<0.001).”

“Though the effect of ML ratio on RTS,S efficacy does not appear to be through anti-CS protein IgG antibody quantity we cannot rule out a role for antibody affinity and other functional properties of the induced response.”

Comment from Advisor:

“Throughout the manuscript it is repeatedly mentioned that 11 sites participated in the phase 2 studies of RTS,S in Africa. In fact, 8 sites were involved in phase 2 studies. Three additional sites (Kenya/Siaya, Malawi and Burkina Faso) joined in at the time of the start of phase 3 work.”

Phase II studies were conducted at eleven geographical sites within 6 countries as outlined below and in a recent publication [6]:

Kenya (2 sites) – Kilifi and Kisumu
Gabon (1 site) – Lambarene
Ghana (1 site) – Kintampo
Mozambique (3 sites) – Manhica, Ilha Josina and Taninga
Tanzania (3 sites) – Korogwe and 2 separate field sites in Bagamoyo
Gambia (1 site) – Upper River Division

REFERENCES


Thanks again for your helpful comments on our manuscript. We hope you will find the revised version acceptable for publication.

Yours sincerely,

George Warimwe