Reviewer’s report

Title: Tolerability and safety of weekly primaquine against relapse of Plasmodium vivax in glucose-6-phosphate dehydrogenase deficient and normal Cambodians.

Version: 1 Date: 4 April 2015

Reviewer: Alan Magill

Reviewer’s report:

General Comments:

• This paper reports results of a well-designed and carefully conducted clinical trial utilizing a treatment regimen for acute vivax malaria of DHA-PIP on days D0, D1, and D2 combined with 0.75 mg / kg primaquine given weekly (1st dose on D0, then weekly X 8) in both G6PDd and G6PDn individuals in three sites in Cambodia.

• The results of the study are important and will inform the appropriate use of primaquine for the radical cure of P. vivax malaria.

• What the results of this paper clearly show are that severely G6PD deficient individuals, those with an enzyme activity of about 1 IU or less / gram of Hgb, cannot safely take a single dose of 0.75 mg / kg. This does not mean that the more moderate deficiency phenotypes cannot take that dose or that even a modified lower mg / kg dose of primaquine given either daily or weekly, cannot be taken by even severely deficient individuals. So the study aim as listed on the bottom of page 4, “generate quality evidence to address the question of whether weekly primaquine could be tolerated…” is not really the question answered by the data.

• Cambodia appears to be rather unique in that a single severely deficient phenotype and genotype (Viangchan) makes up of most of the deficient individuals, at least in the study sites.

• The real contribution in this paper is the carefully monitored outcomes in the 18 individuals with severe G6PDd and could alone be the focus of the report.

Major Compulsory Revisions (these items must be addressed in a revised manuscript):

1. Although the authors are clear on page 3 that primaquine causes a dose dependent AHA, this key fact is not always carried through the manuscript. Bottom of page 4 states the study aim was to generate evidence of safety and tolerability of weekly PQ, the issue is really about the safe dose of PQ for severely deficient phenotypes. In the manuscript ref No. 20 (Everett et al.), the fifteen G6PDd individuals easily tolerated the 15 mg daily dose without any clinical or laboratory sequelae. There was a statistically significant but clinically insignificant decline in hematocrit on day 7. All of the individuals where G6PD was characterized were the G6PDd Mahidol variant with 4-11% of normal
enzyme activity (quantitative enzyme activity in IU / gm Hgb was not reported.) A mg / kg dose was not presented in Everett et al. but one can estimate about some variation around a 0.20 mg/kg daily dose (15 mg in a 70 kg person), so the daily dose in this group is much lower and the enzyme activity is higher than in the current population. Therefore it appears that the 15 mg dose was on the margin of safety so it should be no surprise that a higher dose of 0.75 mg / kg used on the study would cause more toxicity. The statement on page 13 of the discussion, “A G6PDd patient receiving daily primaquine is likely to suffer AHA” is not correct and is in opposition to the dose dependent nature of the problem and as demonstrated in the Everett paper. An A- phenotype can easily take 15 mg (base) daily X 14 days w/o medical supervision or worry as was demonstrated in the 1950s.

2. Page 12, the first paragraph of the discussion is miss leading as written. What the data show is that a severely deficient individual defined as about 1 or less IU / gm of Hgb) cannot take a single dose of 0.75 mg / kg of PQ safely. This is not the same as the weekly regimen. The sentence could be rewritten to insert the words “severely deficient as defined by enzyme activity less than 1 IU / gm Hgb” instead of “G6PDd”. The definition of G6PDd in this paper is < 7.2 IU / gm Hgb. We are pretty confident that the higher class III variants can take this regimen (Alving, Bull WHO 1960) so as written it would not be correct.

3. The definition of G6PD deficiency as a percentage of normal is not well standardized as what is a population normal distribution? Most importantly the key piece of information is not an arbitrary label of enzyme activity that is designated as G6PDd, but rather can the individual safely take the dose of primaquine offered. A severely deficient individual cannot safely take a single dose of 0.75 mg / kg but a moderately deficient individual (3-5 IU / gm Hgb) could (likely) safely take a single dose of 0.75 mg / kg on a weekly schedule, but less likely to be able to take a full 14 day course of 0.50 mg / kg daily X 14 days. The authors should clearly sate that these results apply to severely deficient individuals only at the dose given and cannot extrapolate more broadly to “G6PDd”.

The authors (Page 6) state the population median was 12 IU / gm Hgb from ref 18 and the deficient calculation was 60% of this median, thus anyone with an enzyme activity of < 7.2 IU / gm of HGB would be classified as deficient. However the study population was skewed to the most severely deficient individuals (< 1 IU / GM Hgb). As shown in Fig. 2, the distribution of G6PD activity is 1) very broad, from < 1 to > 18 IU / gm Hgb and also 2) very bimodal with a break around 5-6 IU / gm Hgb.

4. Page 9 and Figure 1, Fig.1 as currently exist is incomplete. The screening failures box only refers to Pailin. Anlong Venh screening failures are not defined. 242 were screened in Anlong Venh and only 10 were enrolled. What happened to the other 232? Of these 10, 9 were deficient. This seems very odd. Same for Veal Veng, Pursat.

5. Page 12, comment that Aspirin is a known hemolytic drug is not supported by the literature. In Abeyraratne et al. (ref No. 27), there is no data on use of ASA in G6PDd rather only a reference to a Table that was derived from previously
published reviews. Many drugs have been falsely implicated in causing AHA in G6PDd because many drugs are used in treating sick people who have g6PDd and are hemolyzing for many reasons. Only carefully performed studies where G6PDd RBCs are labeled and then followed in individuals can determined true drug effect. The world’s expert in this area, Beutler lists ASA as probably safe in G6PDd. (Blood 2008 111(1): 16-24.) The reference to aspirin is not supported by the literature, adds nothing to the data being presented, and thus should be deleted.

Minor Essential revisions (items to consider in a revised manuscript):

1. Page 6, list the manufacturer, brand etc. of the urine pregnancy test used.

2. Page 6, who did and who read the urine color grading chart (manuscript ref No. 22)? The patient, a study staff member? Routinely on day X or only when a patient reported a change in color?

3. Assuring drug quality
   a. Duo Cotexin (Holley) is not a drug approved by a stringent regulatory authority (FDA, EMA, etc.) and is not WHO pre-qualified (http://apps.who.int/prequal/query/ProductRegistry.aspx). What steps and what criteria did the study team take to assure drug quality of the lot used in the study?
   b. Primaquine manufactured by the Government Pharmaceutical Organization, Thailand is not approved by a stringent regulatory authority (FDA, EMA, etc.) and is not WHO pre-qualified. What steps and what criteria did the study team take to assure drug quality of the lot used in the study?

4. The outcomes listed on page 7-8 seem intuitive and reasonable but where did they come from? Were these protocol specified criteria based on expert opinion and experience or some other source(s)? Of the 6 listed, 1-5 are laboratory biomarkers and only No. 6 is a clinical endpoint.

5. Page 8, suggest listing the reference and version of the NIH common terminology criteria for adverse events (CTCAE) used in the study. This is rather complex area and most malaria studies only use selected or modified criteria as CTCAE was developed by NCI for cancer chemotherapy.

6. Page 9, the paragraph starting with “Baseline demographic… is a bit confusing. The comment that there was one patient with wild type genome (as defined by the six PCR sites determined) and a low G6PD activity due to delayed measurement does not make sense. Also a mention of “Four G6PDd patients had missing…,” is followed by 13 had class II and five with class II. There are usually temporal genotype – phenotype mismatches when the two tests are done in acutely ill hemolyzing patients. In this setting it is not important to know the genotype, but rather the phenotype. Does the patient in front of you have enough enzyme activity to safety take the dose of PQ that is being given.

7. Figure 2, it would be useful to the reader to clearly indicate the G6PPDd line (7.2) as defined by the authors on the graph.

8. Page 11, the fractional fall in Hgb on D7 compared to baseline and its relationship to enzyme activity seems quite important and would warrant a figure.
9. Page 11 & table 5, I find the presentation in table 5 confusing. I am used to seeing and thinking about methemoglobin saturation is expressed as the percentage of hemoglobin in the methemoglobin state; as in 1-2% is normal. I am not able to interpret table 5. On page 10 the authors state that no methHb > 4.9% was seen. I understand that. At the dose and regimen used, methemoglobinemia is not an issue. I am not sure Table 5 adds any value or maybe place in supplementary materials.

10. Page 13, paragraph starting with in 2012..., consider adding the most recent (Jan 2015) WHO recommendation (http://www.who.int/malaria/publications/atoz/policy-brief-single-dose-primaquine-pf/en/) and the most recent WHO publication on 8AQ safety (http://www.who.int/malaria/publications/atoz/9789241506977/en/) as refs in addition to or as a replacement for ref No. 30. Also consider changing “would be well tolerated in G6PDd patients” to “would be well tolerated in the severely deficient G6PD patients in Cambodia” and consider changing “the epicenter of artemisinin resistance” to “the epicenter of artemisinin resistant P. falciparum malaria” for optimal clarity. It is extremely important to completely separate in the eyes of the reader the dose of PQ for Pf radical cure and Pv radical cure. These are two entirely separate indications.

Additional comment (just for authors to note, no need to respond unless you want to):

1. Page 3, author’s state that increasing prevalence of CQ resistant vivax blood stage malaria complicates the treatment of vivax malaria. However, the combination of CQ plus an adequate dose of primaquine has been shown to be an effective cure for CQ resistant blood stage Pv. The complexity is that in many locations, Pv radical cure is not attempted or achieved. See a. http://www.ncbi.nlm.nih.gov/pubmed/7769318

2. In this study the authors chose to give the 1st primaquine on D0. In the Clyde paper (manuscript ref No. 16, WHO Bull, 1981), Dr. Clyde recommends giving the 1st PQ after the acute illness is resolving, for example on D4 after dosing of acute drugs. Do the authors think this would make any difference in safety or tolerability?

3. I would be curious to see the enzyme activity plotted against the D0 retic count. In Table 4, the range of D0 retic count in G6PDd is listed at 0.6 to 3.8. For example, did the individual with a retic count of 3.8% have an enzyme activity of < 1 IU / gm Hgb?

4. Page 12, the authors nicely place the observed SAE in context of possible DDI. The literature on DDI and drugs that might cause AHA in G6PDd is confusing and in many cases an association at best. The reality is that sick people take a variety of drugs and supplements and these may or may not cause AHA in G6PDd. Acute illness such as influenza, pneumococcal pneumonia, etc. are common causes of AHA in G6PDd. The severely deficient G6PDd simply have less margin for error. The highest quality evidence for a drug induced AHA came from the radionuclide studies done by Ernie Buetler on survival of labeled
G6PD d RBCs into humans then exposing them to drugs. (nice summary in Beutler E. Glucose-6-phosphate dehydrogenase deficiency: a historical perspective. Blood. 2008 Jan 1;111(1):16-24). Attributions of AHA caused by drugs vary greatly in papers and textbooks often w/o listing the quality of evidence to support such listing. The case report (ref No. 25) is compelling and suggests ciprofloxacin might have contributed to the more significant AHA.

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:

I declare that I have no competing interests