

# Modelling primaquine-induced haemolysis in G6PD deficiency

James Watson<sup>1,2\*</sup>, Walter RJ Taylor<sup>1,2</sup>, Didier Menard<sup>3</sup>, Sim Kheng<sup>4</sup>, Nicholas J White<sup>1,2</sup>

\*For correspondence:

[james@tropmedres.ac](mailto:james@tropmedres.ac) (Mahidol Oxford Research Unit)

<sup>1</sup>Mahidol-Oxford Tropical Medicine Research Unit, Faculty of Tropical Medicine, Mahidol University, Thailand; <sup>2</sup>Centre for Tropical Medicine and Global Health, Nuffield Department of Medicine, University of Oxford, United Kingdom; <sup>3</sup>Unité d'Epidémiologie Moléculaire du Paludisme, Institut Pasteur du Cambodge, 5 Boulevard Monivong - BP 983, Phnom Penh, Cambodia; <sup>4</sup>National Center for Parasitology, Entomology and Malaria Control, Phnom Penh, Cambodia

**Abstract** Primaquine is the only drug available to prevent relapse in vivax malaria. The main adverse effect of primaquine is erythrocyte age and dose dependent acute haemolytic anaemia in individuals with glucose-6-phosphate dehydrogenase deficiency (G6PDd), a common inherited enzymopathy in many areas of the tropics. As testing for G6PDd is often unavailable this limits the use of primaquine for radical cure. A compartmental model of the dynamics of red blood cell production and destruction was designed to characterise primaquine-induced haemolysis using a holistic analysis of all published data on primaquine-induced haemolysis and was used to predict a safer alternative to the currently recommended once weekly 0.75mg/kg regimen for radical treatment in G6PDd patients. The model was fitted to data from a recent study of the effects of weekly high-dose primaquine (0.75 mg PQ base/kg body weight) in G6PD deficient vivax malaria patients from Cambodia. The model suggests that a step-wise increase in daily administered primaquine dose would be relatively safe in G6PDd. If this is confirmed then were this regimen to be recommended for radical cure patients would not require testing for G6PDd in areas where G6PD Viangchan or milder variants are prevalent.

## Introduction

### Radical cure of vivax malaria in G6PD deficient patients

*Plasmodium vivax* accounts for over half the world's malaria burden outside sub-Saharan Africa (Gething *et al.*, 2012). The control and elimination of vivax malaria requires both cure of the blood stage infection (the stage that causes acute illness) and the prevention of later relapses which derive from dormant hypnozoites in the liver (radical cure). Hypnozoites are formed from sporozoites which do not develop immediately following mosquito inoculation but instead remain dormant in hepatocytes for weeks or months before developing and causing recurrent blood stage infections called relapses. In general, *P. vivax* infections in tropical regions are associated with frequent relapses (with intervals as short as three weeks) whilst relapses in *P. vivax* infections from Central America, Northern India and temperate regions are associated with longer intervals from acute infection to first relapse (White, 2011).

Primaquine, an 8-aminoquinoline, is currently the only widely available antimalarial drug for the radical cure of *P. vivax* infections. Primaquine causes predictable oxidant haemolysis in G6PDd, one of the most common genetic abnormalities of man (Cappellini and Fiorelli, 2008). Throughout Asia, the Mediterranean littoral and Africa, allele frequencies for this enzyme deficiency vary between 3 and 35% in the populations at risk from vivax malaria (Howes *et al.*, 2013). As G6PDd has sex-linked inheritance, males are either deficient (hemizygotes) or normal, whereas women can be deficient (homozygotes), normal

42 or partially deficient (heterozygotes) in proportions determined by the Hardy-Weinberg equilibrium. Be-  
 43 cause of Lyonisation, there is substantial variability in the proportion of red cells which are deficient in  
 44 individual heterozygote females (*Beutler et al., 1962*).

45 The degree of haemolysis following primaquine depends on the dose administered and the severity of  
 46 the enzyme deficiency (and in heterozygote females the proportion of erythrocytes which are deficient).  
 47 The more severe G6PDd variants found in SE Asia (e.g. Viangchan, Mahidol, Coimbra, Union) and the  
 48 Middle East/West Asia (e.g. Mediterranean) are generally associated with more severe haemolysis com-  
 49 pared to the common African A- variant. For G6PD normal patients, the primaquine regimen for radical  
 50 cure that is recommended in SE Asia and Oceania (where relapse rates are high) is 0.5 mg base/kg/day  
 51 for 14 days. Elsewhere it is 0.25 mg/kg/day for 14 days. For patients with G6PD deficiency (G6PDd), a  
 52 weekly dose is recommended; 0.75 mg/kg/week given for a total of 8 doses. Unfortunately G6PDd test-  
 53 ing is not widely available despite the recent introduction of point-of-care rapid diagnostic tests (RDTs)  
 54 for G6PDd. These RDTs are currently too expensive to deploy on a wide scale and can be difficult to  
 55 interpret, and thus are not generally available (*Brito et al., 2016; Satyagraha et al., 2016; Oo et al., 2016*).  
 56 Thus, primaquine is commonly not given to patients to avoid the risk of haemolysis so the burden of vivax  
 57 malaria remains high, causing considerable morbidity and economic loss (*Price et al., 2007*).

### 58 **Mechanisms of red blood cell production**

59 The mechanisms regulating red blood cell production and turnover have been well characterised. Red  
 60 blood cells (RBCs) transport oxygen which is reversibly bound to the main red cell protein, haemoglobin.  
 61 RBC production in the bone marrow is regulated to maintain oxygen carrying capacity. When the haemoglobin  
 62 concentration in blood falls, this reduces oxygen carriage and RBC production is up-regulated, a process  
 63 mediated largely by the renal hormone, erythropoietin. At times of increased bone marrow production,  
 64 reticulocytes appear in increased numbers in the circulation (the upper limit of normal is  $\approx 1.5\%$ ). Normal  
 65 RBCs in healthy people have a very stable life expectancy of around 120 days. This is well modelled  
 66 by a Gumbel distribution with low variance. In nucleated cells G6PD can be newly synthesised but red  
 67 cells lose their nucleus before leaving the bone marrow so very young red cells (reticulocytes) have the  
 68 highest G6PD activity and this declines as the RBCs age. In most G6PDd variants, the mutant enzyme  
 69 degrades more rapidly compared to the normal enzyme. Older erythrocytes may have up to five times  
 70 less G6PD activity than reticulocytes. G6PDd results in lowered NADPH and a reduced ability to re-  
 71 generate reduced glutathione. Reduced glutathione protects normal RBCs against oxidant stresses such  
 72 as the haemolytic effects of primaquine metabolites and certain foods, classically fava beans. G6PD is  
 73 also important for the function of catalase, another oxidant defence mechanism. As these non-reusable  
 74 oxidant defence reserves are 'used up', the aging erythrocyte becomes increasingly vulnerable to oxidant  
 75 haemolysis (*Beutler et al., 1954a; Beutler, 2007; Recht et al., 2014*).

### 76 **Evidence from previous studies of oxidant haemolysis in G6PD deficiency**

77 As young red cells have more functional enzyme than older cells, the degree of oxidant haemolysis de-  
 78 pends on the genetic variant of G6PDd and the age distribution of the red cell population. Once the  
 79 older cells have haemolysed, the remaining younger erythrocytes are essentially resistant to further dam-  
 80 age by the same dosing regimen (i.e. drug exposure) (*Beutler et al., 1954a*). However higher primaquine  
 81 doses do cause further haemolysis. This explains the fall then rise in haemoglobin with continued daily  
 82 primaquine administration in mild and moderate severity variants of G6PDd. This temporary primaquine  
 83 insensitivity in G6PDd individuals with continued primaquine administration was characterised by Beut-  
 84 ler and colleagues in a series of studies conducted over sixty years ago (*Beutler et al., 1954a, 1955; Beutler,*  
 85 *1959*) and later exploited by Alving et al to develop the once weekly regimen in G6PDd.

86 By experimenting with high-dose weekly regimens and low-dose daily regimens, Beutler and col-  
 87 leagues showed haemoglobin would first fall as a result of oxidant haemolysis and then rise despite con-  
 88 tinued exposure to the same doses of primaquine which had caused the initial haemolysis. This resulted  
 89 from reactive erythropoiesis (reticulocytosis) that introduced a younger red cell population to the circu-  
 90 lation which was essentially "resistant" to the haemolytic effects of that primaquine dose. Intermittent

91 primaquine administration resulted in progressively smaller cycles of haemolysis followed by reticulocytosis as the red cell population became younger. These results led to a recommendation for a high-dose, 92 once weekly primaquine regimen for radical cure in vivax malaria patients with G6PDd (8 once weekly 93 adult doses of 45 mg) (Alving *et al.*, 1960). This regimen was devised based on studies in subjects with the 94 African A<sup>-</sup> variant of G6PDd, which is one of the mildest deficiencies. Safety was not formally assessed 95 in more severe deficiencies. A recent trial of this regimen in vivax malaria patients with the more severe 96 Viangchan G6PDd variant from Cambodia showed a greater fall in haemoglobin and a delayed recovery 97 from anaemia in G6PDd compared to G6PD normal patients with one patient requiring a blood transfusion 98 (Kheng *et al.*, 2015). These data suggest that weekly primaquine may not be the optimal regimen for 99 the more severe G6PDd variants prevalent outside Africa.

100  
101 Reconsideration of the detailed haematological studies that laid the foundation for the weekly regi- 102 men suggests that an ascending-dose regimen of primaquine, with a schedule that matches the dynamics 103 of red blood cell production, could induce a safe ‘slow burn’ haemolysis, even in individuals with severe 104 G6PD variants, and would still deliver a total therapeutic dose for radical cure.

105 Accordingly, our study had two objectives; first, to construct a compartmental model for red blood 106 cell dynamics which could be used to analyse all available data from past studies of haemolysis in G6PDd 107 individuals, and second to predict an optimal ascending dose regimen which would be safe and efficacious 108 yet practical and could, therefore, be recommended without G6PD testing.

## 109 Methods

### 110 Mathematical model

111 The structure of the model of red cell dynamics is similar to the compartmental model developed by Savill 112 *et al.* (2009). RBC dynamics are simulated by tracking the age distribution of the red blood cells in hourly 113 blocks. The homeostatic dynamics which maintain the number of red blood cells or haematocrit at a 114 steady state are straightforward. At steady state, approximately 0.83% of RBCs are replaced each day 115 and 1% of RBCs in the circulation are reticulocytes. Severe acute anaemia has two consequences in the 116 bone marrow. Reticulocytes are released into the circulating blood at an earlier age and with increased 117 erythropoiesis normoblasts may be released into the circulation (reported as nucleated RBCs) (Hillman, 118 1969). Previous iron turnover studies in humans following phlebotomy suggest sigmoid relationships for 119 both of these processes (Hillman, 1969).

### 120 Compartmental model of RBC dynamics

121 The steady state haemoglobin concentration is denoted Hb\*. The time at which reticulocytes are released 122 into circulation is a function of the haemoglobin concentration at time t, denoted Release(Hb<sub>t</sub>). At steady 123 state, when Hb<sub>t</sub> = Hb\*, the reticulocytes mature in the bone marrow for 3.5 days (i.e Release{ Hb\* } = 3.5) 124 and then spend approximately one day in the circulation before becoming erythrocytes. In anaemia, 125 release can occur after only one day (then the cells are reticulocytes for 3.5 days in the circulation). These 126 relationships were shown from plasma iron turnover studies following phlebotomy (Hillman, 1969).

127 By modelling the number of circulating RBCs directly, it is possible to compute the haemoglobin 128 concentration at each time point in the simulations. The steady state number of RBCs corresponds 129 to steady state haemoglobin and steady state haematocrit. The relationship between haematocrit and 130 haemoglobin is assumed to be linear (Lee *et al.*, 2008). At steady state the body produces approximately 131 10<sup>8</sup> RBCs per hour. In the model, this is used as the baseline production quantity, represented by a 132 production factor of  $\rho = 1$ . In extreme anaemia this can be increased fivefold or more, e.g.  $\rho \geq 5$ . Both 133 Release(Hb<sub>t</sub>) and  $\rho(\text{Hb}_t)$  are modelled as sigmoid functions:

$$134 \quad \rho(\text{Hb}_t) = \frac{\rho^{\max}}{1 + e^{\lambda(\text{Hb}^*)(\text{Hb}_t - \text{Hb}_{50}^R)}} \\ \text{Release}(\text{Hb}_t) = 1 + \frac{2.5}{1 + e^{-k(\text{Hb}_t - \text{Hb}_{50}^R)}}$$

Parameter	Units	Meaning
$d$	unitless	Parameter of age-dependent killing function.
$Hb^*$	Hb	Steady state haemoglobin concentration.
$Hb_t$	Hb	Haemoglobin concentration at time $t$ .
$\rho(Hb)$	unitless	Fold-increase in production of RBCs as a function of haemoglobin concentration (at steady state $\rho(Hb^*) = 1$ ).
$\rho^{\max}$	unitless	Maximum fold increase in RBC production, this will be reduced in anemia.
$Hb_{50}^{\rho}$	Hb	Haemoglobin concentration for which production is elevated to $\rho^{\max}/2$ .
$\text{Release}(Hb_t)$	days	Time of release of reticulocytes into circulation as a function of haemoglobin concentration.
$k$	unitless	Hill coefficient of sigmoid function $\text{Release}(Hb_t)$ .
$Hb_{50}^R$	Hb	Haemoglobin concentration corresponding to mid-point of sigmoid describing reticulocyte release into circulation.
$T_{\min}$	hours	Earliest age of a RBC vulnerable to primaquine-induced haemolysis.
$T_{\text{lag}}$	hours	Time to reach the maximum haemolytic effect of primaquine.

**Table 1.** Parameters and functions of the compartmental model along with their interpretation.

135 with  $\lambda(Hb^*)$  given by:

$$\lambda(Hb^*) = \frac{\log(\rho^{\max} - 1)}{Hb^* - Hb_{50}^{\rho}}$$

136 where  $Hb_t$  is the haemoglobin concentration at time  $t$  (or equivalently the haematocrit);  $\rho^{\max}$  is the maximum fold increase in steady state RBC production;  $Hb^*$  is the steady state haemoglobin concentration;  $Hb_{50}^{\rho}$  is the mid-point of the  $\rho$  sigmoid function (the haemoglobin concentration at which production is equal to  $\rho^{\max}/2$ ;  $k$  the Hill coefficient which regulates the steepness in response to perturbations in haemoglobin levels;  $Hb_{50}^R$  the mid-point of the release function.

141 For simplicity it is assumed that in the normal healthy state all red blood cells live exactly 120 days as erythrocytes, and therefore these two functions are sufficient to model the feedback loops which regulate perturbations to haemoglobin levels.

144 The following class of functions is used to model the red cell age-dependent primaquine-induced haemolysis:

$$\text{Probability (PMQ induced death at age } t) = \begin{cases} e^{-(t-120)^d} & t \in [T_{\min}, 120] \\ 0 & t \leq T_{\min} \end{cases} \quad (1)$$

146 where  $T_{\min}$  is the age of the youngest red blood cells lysed by primaquine. This parameter varies as a function of the degree of G6PD deficiency (determined by the genetic variant of G6PDd). The data from *Pannacciulli et al. (1965)* suggest cells as young as 16 days can be lysed with a daily primaquine dose of 30mg in the severe Mediterranean variant, whereas in the less severe African A- variant haemolysis appears confined to cells older than 50 days (*Beutler et al., 1954a*). The steepness of this 'killing function' is regulated by the parameter  $d$ , with smaller values of  $d$  giving a sharper drop in haemoglobin levels.

152 Depending on the degree of severity of G6PD deficiency, haemolysis will be observed more or less quickly after the first dose of primaquine. To simulate this effect, a time lag component is added to the model, the value of which will depend on the genetic variant of G6PDd. The time lag component, denoted  $T_{\text{lag}}$ , reduces the total haemolytic effect (as given in equation 1 for the first  $T_{\text{lag}}$  hours after the first dose of primaquine. A glossary of all parameters of the model alongside their units and interpretation is given in Table 1.

158 Available data on primaquine induced haemolysis are sparse (these data are reviewed in detail in the  
 159 next section). Thus many of the free parameters such as  $T_{lag}$  and  $T_{min}$  are fixed using expert opinion  
 160 and their impact on the modelling evaluated by a sensitivity analysis shown in appendix 1. A primary  
 161 goal of this analysis was to parametrize the relationship between primaquine dose and haemolysis for  
 162 a given severity of G6PD deficiency. Thus the dose-response curve varied with the different genetic  
 163 mutations. A preliminary analysis (Figure 5) of the available historical data (Figures 1 & 2) suggests that the  
 164 relationship between dose and age-dependent haemolysis as parametrised by the function in equation  
 165 1 is logarithmic:

$$\log d = \alpha PMQ_{dose} + \beta x + c$$

166 where  $x$  are individual covariates of importance such the G6PDd variant and sex (the deficiency is X-  
 167 linked) and  $PMQ_{dose}$  is the dose of primaquine.

### 168 Inputs and Outputs of model

169 The goal of this compartmental model was to simulate haemolysis following primaquine administration  
 170 to a G6PDd individual over a fixed period of time. In order to do this, the model needs as inputs the  
 171 following elements.

- 172 1. The age distribution of RBCs both in the bone marrow (normoblasts and reticulocytes) and in circu-  
 173 lation (reticulocytes and erythrocytes). This is represented as a vector of counts of RBCs for each  
 174 age group discretized into hourly blocks.
- 175 2. The number of hours for which to simulate the model forward in time. This includes a dosing sched-  
 176 ule (binary vector discretized in hourly blocks where 1 represents drug in body and 0 represents  
 177 drug absent). In all the simulations shown, the drug schedules are designed in multiples of 24 hour  
 178 blocks (the terminal elimination half-life of primaquine is  $\approx 5$  hours). In this manner it is possible to  
 179 simulate weekly dosing and daily dosing.
- 180 3. The dosing level of the drug (e.g. in mgs). This is defined by the value of parameter  $d$  from equation  
 181 1.

182 The simulations output the haemoglobin concentration over time, the reticulocyte percentage over  
 183 time, and the final age distribution of RBCs in both the bone marrow and in circulation.

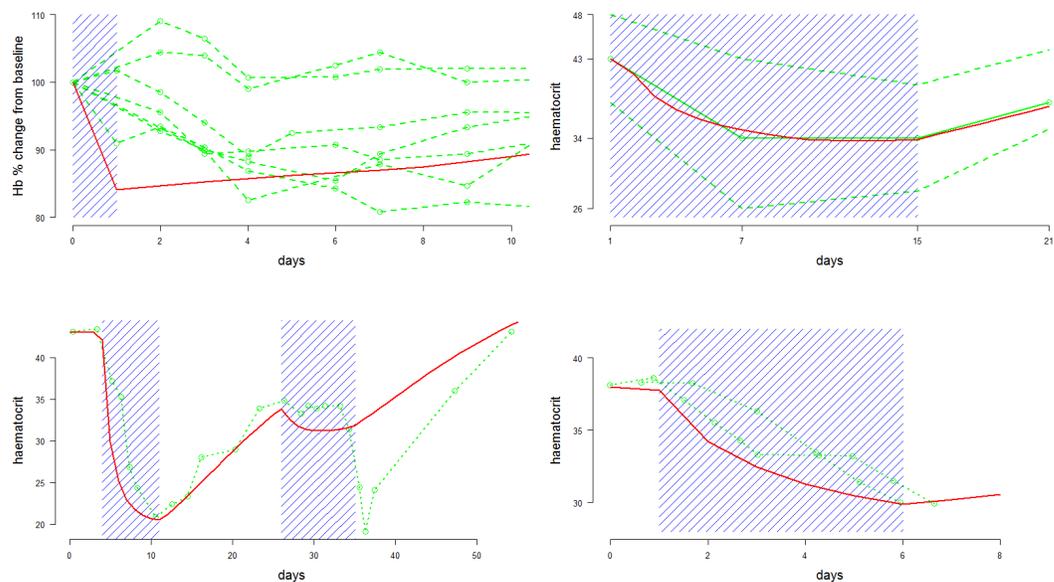
184 The structure of the model also allows for the simulation of the effect of malaria within an individual by  
 185 altering the age distribution as *P. vivax* invades young erythrocytes exclusively. It is assumed that healthy  
 186 individuals with no history of haemolytic events in the last 4 months will have a uniform age distribution  
 187 of RBCs. Simulations in *P. vivax* infections could be done by shifting the age distribution. Model code  
 188 is available in the supplementary materials. Supplementary File 1 provides instructions for running the  
 189 code. Supplementary File 2 provides posterior MCMC samples from the model.

## 190 Data

### 191 Historical studies

192 Although primaquine was first tested in humans in 1944 and approved by the US FDA in 1952, there are  
 193 very few precise data on its haemolytic effect in G6PDd individuals. Most of the studies only present  
 194 sparse data, often limited to summary statistics, and with small sample sizes ( $n \approx 2$ ). This section presents  
 195 an analysis of the most information-rich studies conducted over the last 60 years and shows how they can  
 196 be used to design informative prior distributions for the compartmental model. An exhaustive review of  
 197 all available data on the haemolytic effects of primaquine has been reported recently by *Recht et al. (2014)*.  
 198 There are however only five studies which present useful data on falls in haemoglobin concentrations  
 199 over time. Throughout this section, for consistency in Figures 1-4, data are shown in green and model  
 200 fits/predictions in red.

201 The data extracted from past studies in healthy volunteers are shown in Figures 1 & 2. These figures  
 202 compare least squares model fits with the data. The studies span four different variants of G6PD de-



**Figure 1.** Comparison between approximate model fits (red) and data (green) extracted from four primaquine studies with single dose or daily regimens all at 30/45mg adult doses. Dosing periods are shaded in blue. The top two plots are for Mahidol and Viangchan variants, respectively. The bottom two plots are for the Mediterranean variant. From top left to bottom right: single 45mg dose given to 7 G6PDd Mahidol Thais (Charoenlarp *et al.*, 1972); 14 daily doses of 30mg given to 15 G6PDd presumed Viangchan variant Khmer soldiers (only mean and extreme values reported) (Everett *et al.*, 1977); 1 G6PDd Med Sardinian given two courses of daily 30 mg doses (Pannacciulli *et al.*, 1965); 2 G6PDd Med Sardinians given 5 daily doses of 30mg (Salvidio *et al.*, 1967)

203 ficiency: Mediterranean, Mahidol, Viangchan<sup>1</sup>, and African  $A^-$ . These can be categorised generally as  
 204 severe, moderate, moderate and mild variants of G6PDd, respectively.

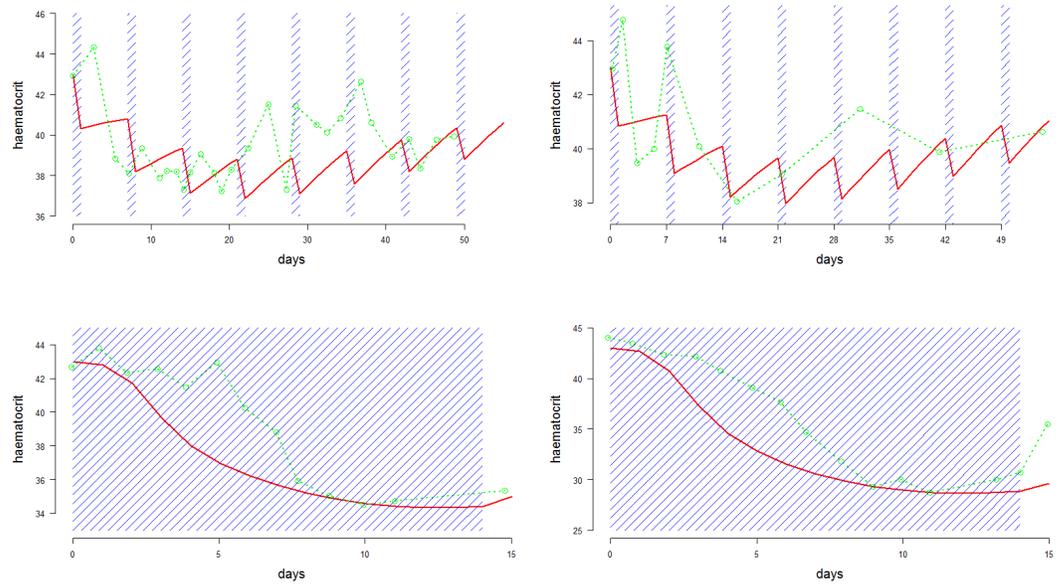
205 The deterministic compartmental model reproduces the essential patterns of the observed dynamics,  
 206 namely, a more rapid decrease in haematocrit at the start of the regimen which then slows as the haemol-  
 207 ysis becomes self-limiting. However, some aspects cannot be reproduced by the model. The bottom left  
 208 plot of Figure 1 shows a sharp drop in haematocrit after the second round of primaquine administration.  
 209 This is surprising, although the fact that it is outside the administration period may be an error in plotting  
 210 in the original paper. The compartmental model cannot reproduce such a marked second drop after an  
 211 initial fall of over 50% from baseline. In Figure 2, for the bottom two plots, the model predicts a faster  
 212 initial decrease than observed for a similar nadir drop.

213 These data were used to select a plausible range of values for the parameter  $d$  in equation 1. This  
 214 parameter governs the age-dependent haemolysis at different dosing levels.

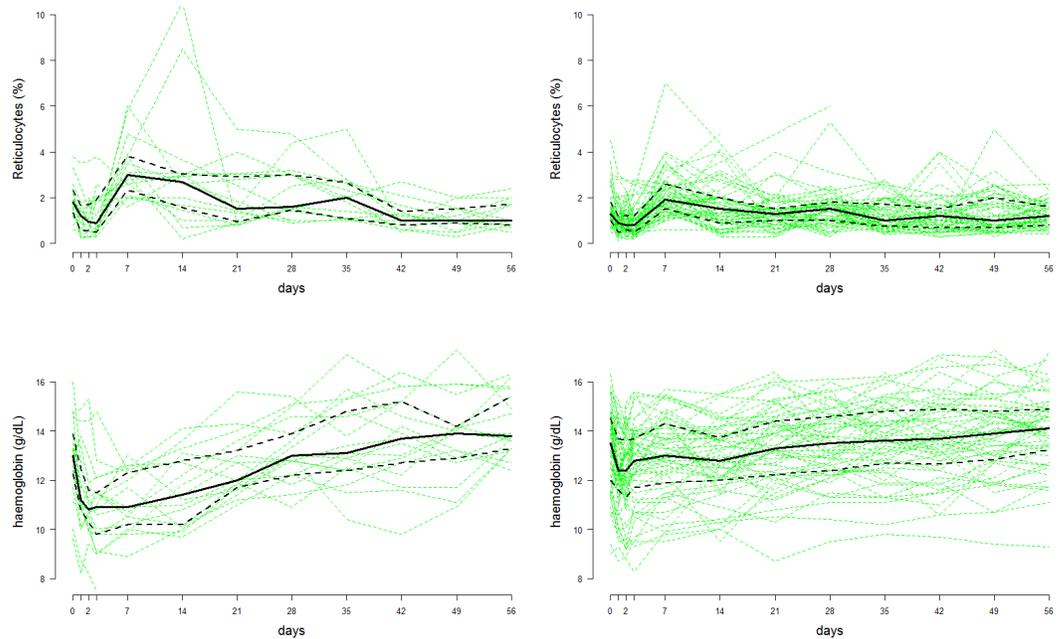
#### 215 Weekly high-dose primaquine in G6PDd Viangchan

216 The largest and most recent study of primaquine-induced haemolysis in G6PD deficiency is from *Kheng*  
 217 *et al.* (2015). In this study, 75 Cambodian patients with vivax malaria were given primaquine 0.75 base  
 218 mg/kg weekly for 8 weeks. Of these, 17 were G6PDd Viangchan (14 homozygous males and three  
 219 heterozygous females) and 1 was a homozygous male with G6PDd Canton. Haemoglobin concentrations  
 220 and reticulocyte counts were measured on days 0,1,2,3 and subsequently each week, before the next  
 221 dose of primaquine. One patient had a marked drop of haemoglobin falling to 7.5 g/dL and required a  
 222 blood transfusion. The data are shown in Figure 3. The variation in the measurement of haemoglobin  
 223 was approximately 1 g/dL. For reticulocyte counts the measurement error is much greater when done by  
 224 microscopy (i.e. counting per 100 red blood cells).

<sup>1</sup>The Khmer soldiers whose data are shown in Figure 1 are incorrectly referred to by Everett *et al.* (1977) as G6PDd Mahidol. They are in fact most likely to be G6PDd Viangchan, as the variant was discovered some ten years later (1988).



**Figure 2.** Comparison between approximate model fits (red) and data (green) extracted from four primaquine studies on the same individual with G6PDd African A<sup>-</sup> (Alving et al., 1960). Dosing periods are shaded in blue. The top two plots are for weekly dosing regimens (8 doses): left is 60mg per week; right is 45mg per week; the bottom two plots are daily dosing regimens (14 doses): left is 15mg per day; right is 30mg per day.



**Figure 3.** Time series data of reticulocyte count (top row) and haemoglobin concentrations (bottom row) from the Cambodian study on G6PDd individuals ( $n = 18$ , left column) and G6PD normals ( $n = 57$ , right column) (Kheng et al., 2015). The faint green lines show individual patient data; the thick black lines represent the population median values at each time-point; the dashed black lines show the interquartile range.

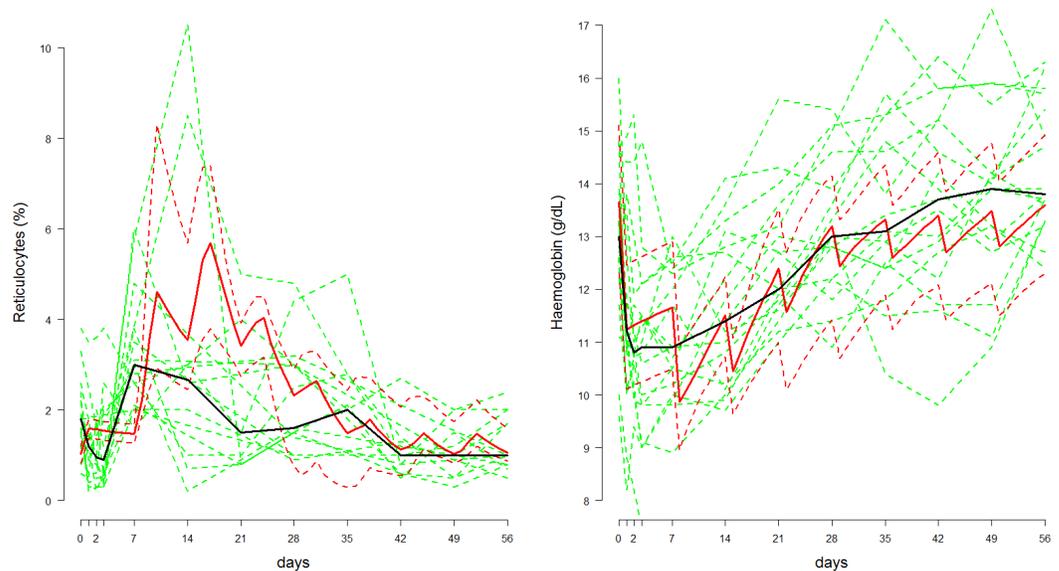
## 225 Model fitting using MCMC

226 The historical (Figures 1 & 2) and Kheng (Figure 3) data were used to fit the compartmental model; the  
 227 former were used to select suitable prior distributions for parameters. Bayesian model fitting via MCMC  
 228 was then applied to the data from the weekly high-dose primaquine in Cambodia (*Kheng et al., 2015*).  
 229 The likelihood of the parameters is defined by a deterministic simulation from the compartmental model  
 230 for a given dosing regimen and assumes both the haemoglobin levels and reticulocyte counts are ob-  
 231 served with Laplace distributed errors<sup>2</sup>. A Bayesian hierarchical structure was used for the steady state  
 232 haemoglobin and the maximum increase in the production of red blood cells. This makes the assumption  
 233 that each patient in the study is characterized by an individual steady state haemoglobin concentration  
 234  $Hb^*$  and a maximum production capacity  $\rho^{\max}$  drawn from a population distribution (normal distributions  
 235 in both cases). Weekly informative priors were used for all parameters and the posterior distribution  
 236 was estimated using MCMC with a Metropolis-Hastings proposal. Details of prior distributions and his-  
 237 tograms of posterior distributions, together with convergence diagnostics and summary statistics are in  
 238 the Appendix 1, 'Structure of hierarchical model and MCMC diagnostics'.

## 239 Results

### 240 Model fit

241 Figure 4 shows hypothetical data simulated from the compartmental model with a primaquine regimen  
 242 of 45mg weekly for 8 weeks in adult G6PD deficient Cambodian patients and with parameters randomly  
 243 drawn from the Bayesian posterior distribution.



**Figure 4.** Comparison between the data from *Kheng et al.* (shown in green, population median in thick black line) and posterior predictive 80% credible intervals (shown in red, median: thick line; 10&90% boundaries: dashed lines) in which adult Cambodian patients who were G6PD deficient were given weekly primaquine (45mg) for 8 weeks. *Left:* reticulocyte response; *Right:* haemoglobin response.

244 The signal-to-noise ratio in the reticulocyte data is low and this is apparent from the median reticulo-  
 245 cyte count which varies considerably during the 56 days.

246 In comparison, simulations from the mechanistic model show that a rise in the reticulocyte count  
 247 should occur approximately one week after the first dose and then return to normal slowly over the  
 248 subsequent six weeks. The serial haemoglobin data on the other hand show a clear trend with a large fall

<sup>2</sup>Assuming Laplace errors is equivalent to minimizing absolute deviation.

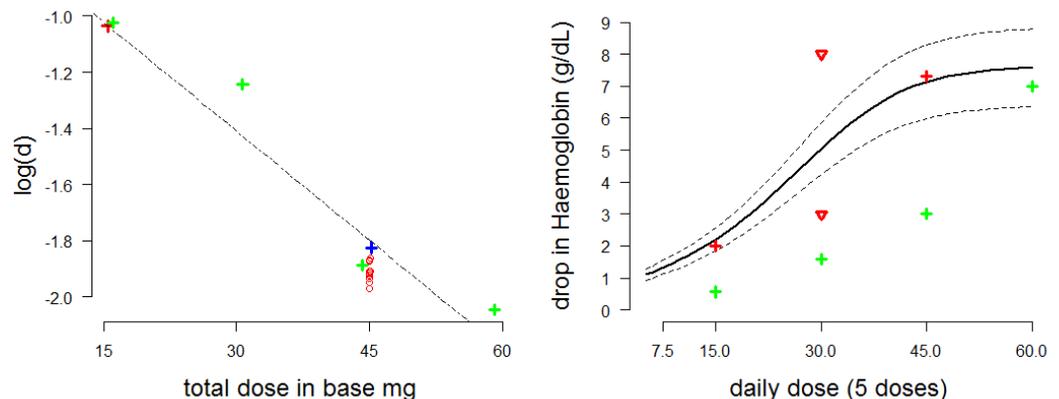
249 after the first dose, a smaller fall after the second and then a gentle recovery with no major effect from  
 250 subsequent primaquine doses. This trend is reproduced by the model and the posterior distribution also  
 251 characterises satisfactorily the variance observed in steady state haemoglobin concentrations.

### 252 Predicted dose response

253 Combining the data from Figures 1, 2 and 3, it is possible to estimate a primaquine dose-haemoglobin  
 254 response curve for G6PDd individuals whose severity is similar to the 'moderate severity' variants G6PDd  
 255 Mahidol/Viangchan. The data at different dosing levels are sparse and the studies have been done in very  
 256 different contexts; however, the strong mechanistic assumptions used to construct the compartmental  
 257 model regularize the problem enough to compare the studies in a principled way. The data from G6PDd  
 258 Mediterranean are excluded from this dose-response curve estimation because the haemolysis observed  
 259 with this variant is considerably greater than for G6PDd Mahidol/Viangchan. However, the observed  
 260 falls in haemoglobin after 5 daily doses of 30mg in G6PDd Med Sardinians are shown by the red triangles  
 261 in Figure 5, right plot, for comparison.

262 The posterior MCMC samples inferred from the Kheng data can be used to approximate model un-  
 263 certainty around the median dose-response curve. The right plot of Figure 5 shows the posterior pre-  
 264 dictive dose-response curve with 90% credible intervals, where the 'response' is defined as the drop in  
 265 haemoglobin after 5 days at a given dosing level. Overlaid are estimates of the falls in haemoglobin in-  
 266 duced by 5 daily doses from studies in Figures 1 & 2, and an extrapolated estimate from the posterior  
 267 distribution of the model fitted to data from weekly dosing in Viangchan variant.

268 It is of interest to compare the fitted dose-response relationship in Figure 5 (right: thick black line) -  
 269 corresponding to the more severe variants of G6PDd - with the green crosses corresponding to observed  
 270 and fitted haemolysis in G6PDd African  $A^-$  (mild variant). As would be expected, for the mild variant the  
 271 dose-response relationship has the same shape but is shifted to the right.



**Figure 5.** Estimating the dose-response curve for moderate/severe G6PDd. Left: estimates of the  $\log d$  parameter as a function of administered dose plotted with a linear regression curve (red cross: Viangchan; red circles: posterior estimates from model fitted to data from G6PDd Viangchan; blue cross: Mahidol; green crosses: African  $A^-$ ). Right: dose-response curve (thick black line) with 90% credible intervals (dotted black lines) as measured by fall in haemoglobin ( $y$ -axis) after 5 days at a given dose ( $x$ -axis) based on draws from the posterior distribution. The red and green crosses are the estimated falls after 5 days from Viangchan and African  $A^-$  studies, respectively (see Figures 1 & 2). The red triangles show the falls observed in G6PDd Med studies from Figure 1.

### 272 Safe optimal regimen

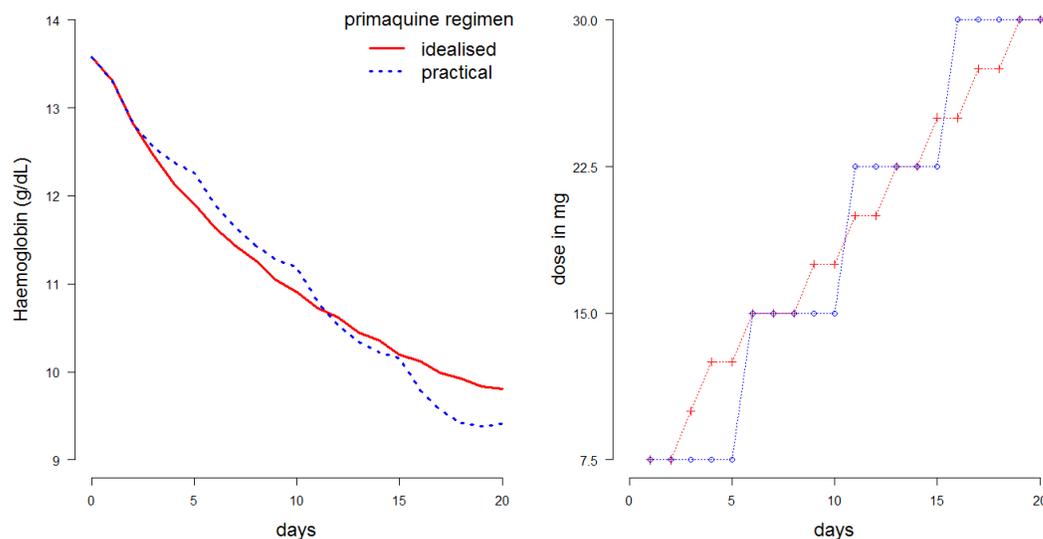
273 The currently recommended dose for the radical cure of vivax malaria in an adult in SE Asia and Oceania  
 274 delivers 420mg (i.e. 30 mg/d x 14 d) of primaquine and is very effective (*John et al., 2012*). The maximum  
 275 primaquine dose administered in the weekly regimen is 360 mg (8 x 45 mg) but the efficacy of this regimen

276 has only been reported in Afghan refugees in Pakistan, a country with a relatively low relapse rate (*Leslie*  
277 *et al., 2008*).

278 The primary objective of our research is to design a novel primaquine regimen that could be given  
279 safely to individuals with G6PDd or of unknown status without G6PD testing and deliver a total dose  
280 that would be efficacious. The scientific hypothesis is that the same total dose could be given safely with  
281 tolerated declines in Hb over a longer duration by starting with a lower initial dose which is increased  
282 gradually over time. The ascending dose regimen would allow for a steady adjustment of the age distri-  
283 bution of RBCs by both slow primaquine-induced haemolysis and the resulting increased erythropoiesis.  
284 These results only concern ascending dose regimens given over 20 days. There are two reasons for this;  
285 first, adherence to long course regimens is likely to be poor, and second, the first relapses emerge from  
286 the liver about 14 days after starting treatment so the primaquine regimen has to provide sufficient drug  
287 to prevent emergence or eliminate these parasites.

288 *Definition* For practical purposes, an acceptable ascending dose regimen is defined as a monotonic  
289 increasing dose regimen satisfying the following conditions: (i) the total dose is  $>380\text{ mg}^3$  (ii) every incre-  
290 ment is a multiple of 2.5mg, (iii) the minimum adult daily dose is 5 mg, and (iv) the maximum adult daily  
291 dose is 30 mg.

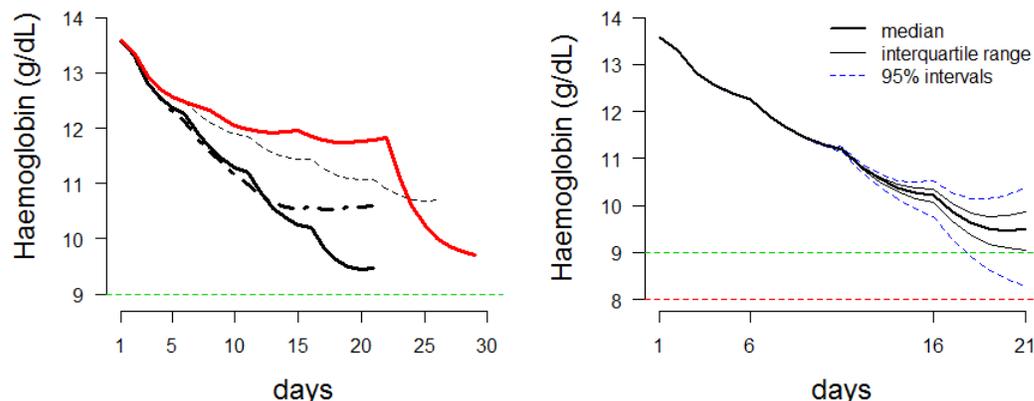
292 The optimal ascending-dose regimen is defined as the one resulting in the slowest haemolysis, where  
293 the rate of haemolysis is penalized by the squared gradient. The optimisation problem is non-convex  
294 for all ascending dose regimens, so the solution is approximated using a greedy search algorithm. An  
295 estimated optimal dosing regimen satisfying the criteria defined above is shown in Figure 6, plotted in  
296 red (left: haemolytic effect; right: daily dosing of the ascending regimen). This was found using the  
297 median Bayesian posterior parameter estimates and a dose-response relationship taken from a linear  
298 interpolation of all points in Figure 5 (left plot). In blue is a simplified version of this ascending-dose  
299 regimen, broken into four 5-day cycles at a fixed dose. The resulting haemolysis from the blue regimen is  
300 greater and the drops in haemoglobin are more irregular (left plot). Video 1 in the supplementary materials  
301 illustrate the red blood cell dynamics over the course of this regimen.



**Figure 6.** Comparison of two 20-day ascending-dose regimens. *Left:* haemolysis over time resulting from regimens. Blue: simplified regimen; red: idealized optimal regimen. *Right:* daily dosing construction for the two regimens. Total dose of blue regimen is 375mg; total dose of red regimen is 382.5mg.

<sup>3</sup>Current tablet sizes do not allow for a regimen to provide 420 mg easily to all adult patients – and 380mg is considered to give similar efficacy.

302 Although intuitively one might think that starting with a lower dose was safer, this regimen is actually  
 303 the worst choice. This is because it delivers too little primaquine at the start (observe very small decreases  
 304 in haemoglobin concentration) with a reticulocyte response that is too weak to render the RBCs “resistant”  
 305 to primaquine; the necessity to increase the PQ dose too fast to compensate for the slow start and to  
 306 deliver an efficacious total dose results in a large drop in Hb on day 22.



**Figure 7.** Dynamics of ascending regimens. *Left:* Comparing the haemolytic effect of four regimens. Thick black line: proposed optimal regimen; thick black dashed line: more conservative regimen with lower total dose; thin black dashed line: longer duration regimen for more severe variants; thick red line: bad choice regimen. *Right:* Posterior predictions for the proposed ascending dose for a given starting haemoglobin (steady state). Prediction using the median posterior values is shown by thick black line. Predictions for 100 random draws from the posterior are shown by dashed blue lines. The horizontal line at a haemoglobin concentration of 9 is a proposed conservative ‘safety threshold’. Horizontal line at a haemoglobin concentration of 8 is a proposed limiting toxicity threshold.

Regimen	Day					
	1-5	6-10	11-15	16-20	21-25	26-30
A	7.5mg	15mg	22.5mg	30mg	-	-
B	7.5mg x3d;10mg x 2d	15mg x 3d; 17.5mg x 2d	20mg	22.5mg x 3d; 25mg x 2d	-	-
C	5mg	10mg	15mg	20mg	25mg	-
D	5mg	5mg x 2d; 10mg x 3d	10mg x 4d; 15mg x 1d	15mg	15mg x 1d; 30mg x 4d	30mg x 3d

**Table 2.** Illustrative regimens. A is our proposed optimal ascending dose regimen; B is a slight variation on this regimen (accelerated); C is a slower ascending dose regimen (potentially suitable for more severe variants); D illustrates a very poor regimen.

### 307 Discussion

308 Primaquine is widely recommended for the radical cure of vivax malaria but it is often not given because  
 309 testing for G6PD deficiency is not widely available outside large centres. This has deleterious conse-  
 310 quences for vivax malaria affected communities because it is the multiple relapses of vivax malaria from  
 311 liver hypnozoites that cause substantial morbidity.

312 Seminal research conducted over 50 years ago characterized the biology of oxidant haemolysis caused  
 313 by primaquine and provided an alternative once weekly regimen for patients who were G6PD deficient  
 314 based on controlled haemolysis. This was shown to be safer in adult subjects with the “mild” African  
 315 A<sup>-</sup> variant of G6PD deficiency, but was recommended for all G6PD variants with variable adoption by  
 316 countries since. In some countries (e.g. Iran) it is the standard radical treatment for all patients. The safety  
 317 and effectiveness of the high dose weekly regimen have been studied little over the past five decades.

318 Uncomplicated malaria treatment recommendations are usually a trade-off between dosing precision  
 319 and operational feasibility. A regimen which is long or complicated may be adhered to poorly. In this

320 particular case it must also be able to prevent or suppress relapsing *P. vivax* or *P. ovale* parasites which  
 321 begin to emerge from the liver as early as two weeks (becoming patent about one week later) in SE  
 322 Asia and Oceania. This modelling exercise, based on all available data, sought to devise a primaquine  
 323 regimen which would be safer in G6PDd patients, and, therefore, might be deployed without G6PD  
 324 testing. It was calibrated against recent data in Cambodian patients most of whom had the Viangchan  
 325 G6PD variant. Thus, the model predictions of the degree of haemolysis and the tolerability and safety  
 326 profile would be expected to hold for variants with similar or less severe enzyme abnormalities, but it  
 327 would not necessarily hold for more severe variants such as G6PD Mediterranean where more clinical  
 328 research is required.

329 Under all circumstances, the ascending regimen proposed here would be expected to be safer than  
 330 the current 14 day regimens in G6PD deficient hemizygous males and homozygous females, especially  
 331 the 0.5 mg/kg regimen needed for frequent relapsing *P. vivax*. This is clinically relevant also for female  
 332 heterozygotes. Even with current rapid testing methods (e.g. fluorescent spot test and RDTs) which  
 333 generally detect patients with  $\leq 30\%$  normal G6PD activity, the haemolytic risk in heterozygote females,  
 334 who may be classified erroneously as "G6PD normal", could still be substantial. Up to  $\approx 70\%$  of their  
 335 erythrocytes may be G6PD deficient, and clinically significant haemolysis may result from daily higher  
 336 dose primaquine regimens given to female heterozygotes (Chu et al., 2017).

337 Although this compartmental model of RBC dynamics is highly simplified, it reproduces the essen-  
 338 tial dynamics of the body's response to primaquine-induced haemolysis in both healthy individuals and  
 339 malaria patients. It can therefore help to guide the design of a Phase I study to evaluate its predictions,  
 340 and thereby develop an optimal ascending dose regimen of PQ. An adaptive design protocol has been  
 341 developed to test the simplified regimen (A) in G6PDd Mahidol healthy volunteers. A study in healthy  
 342 G6PD deficient volunteers is essential to characterise the haemolytic response. Data from such a study  
 343 can then be used to determine an optimal regimen which would then be tested for safety, and efficacy  
 344 (i.e. radical cure) in vivax malaria patients in a Phase II (i.e. to define the PK-PD relationship in patients).  
 345 Whether patients would adhere sufficiently to a longer regimen is an important operational concern so  
 346 the optimised regimen would then need to be assessed for safety and effectiveness in larger field trials.

347 This use of mathematical modelling such as this could also be readily applied to the slowly eliminated  
 348 8-aminoquinoline tafenoquine, currently being tested for safety and efficacy in humans (Beck et al., 2016).  
 349 Tafenoquine has the great advantage of being administered as a single dose for radical cure due to its long  
 350 terminal elimination half-life. However, this means it could be dangerous in G6PD deficiency. Whereas  
 351 the rapidly eliminated primaquine can be stopped if there is significant haemolysis, limiting the haemolytic  
 352 effect, the haemolytic effect of the slowly eliminated tafenoquine cannot be readily reversed and so  
 353 haemolysis will continue until all susceptible red cells are destroyed. Combined regimens for G6PDd  
 354 patients in which primaquine is given initially to induce controlled haemolysis followed by tafenoquine  
 355 might be possible, and would allow shorter total treatment durations. Data on the Hb response to differ-  
 356 ent doses of tafenoquine would be necessary to calibrate the model.

357 The results of this study show how care will need to be taken when designing an ascending primaquine  
 358 dose regimen in order to minimize falls in haemoglobin. This is shown by the toxic regimen D (Table 2 and  
 359 Figure 7). This gives an insight into the 'memory' property of the ascending dose regimens. The effect  
 360 of a 30 mg dose will entirely depend on which doses were given during the previous days. In conclusion,  
 361 these results suggest that an ascending PQ dosing regimen for vivax radical cure might be well tolerated  
 362 and effective in mild or moderately severe G6PDd variants. These predictions should now be tested in  
 363 an adaptive phase I study.

### 364 **Supplementary Materials**

365 **Figure 3 - source data 1:** this provides the source data for the reticulocyte counts and haemoglobin  
 366 concentrations over time from the Kheng et al. (2015) study on weekly high-dose primaquine.

367 **Supplementary File 1:** instructions for running the model code provided in source code.

368 **Supplementary File 2:** posterior MCMC samples from model run on data from Kheng et al. (2015).

369 **Video 1:** animated video showing the red blood cell dynamics for our optimal ascending dose regimen.

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## Appendix 1

### Structure of hierarchical model and MCMC diagnostics

The analysis from section 'Model fitting' assumes that the parameters  $d, k, \text{Hb}_{50}^{\rho}, \text{Hb}_{50}^R$  are defined on a population level (same for all individuals in the study). The parameters  $\text{Hb}_i^*, \rho_i^{\max}$  are defined on the individual level but drawn from a population distribution. Thus the  $i^{\text{th}}$  patient is characterized by:

$$\begin{aligned}\text{Hb}_i^* &\sim N(\theta_{\text{Hb}}, \sigma_{\text{Hb}}^2) \\ \rho_i^{\max} &\sim N(\theta_{\rho}, \sigma_{\rho}^2)\end{aligned}$$

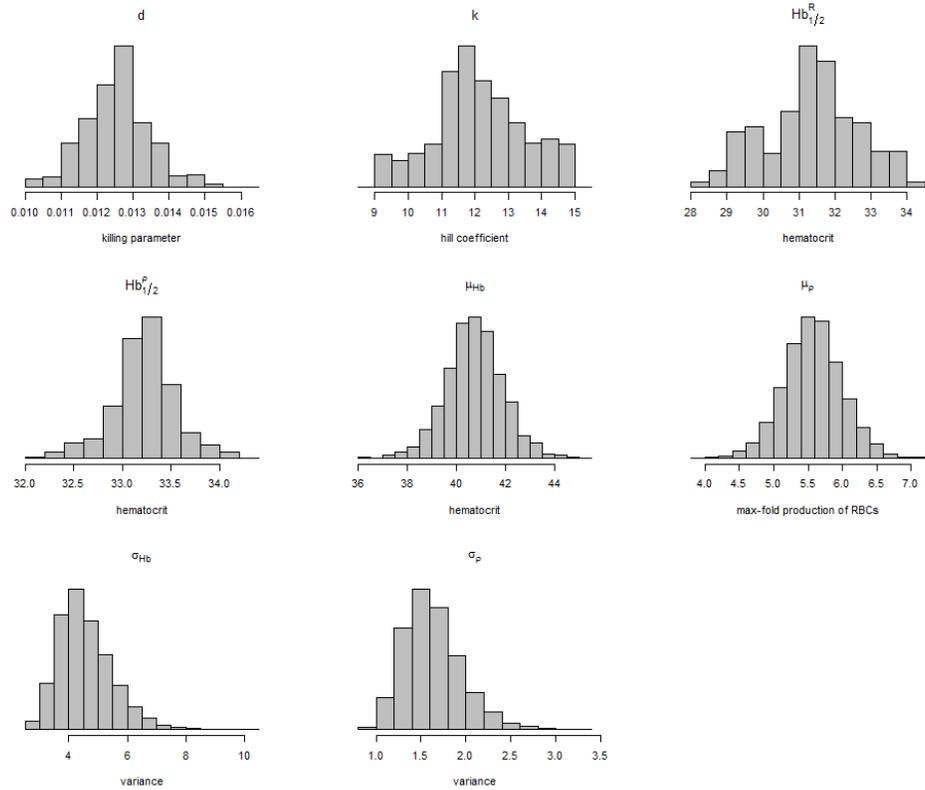
We set informative priors on the hyperparameters  $\theta_{\text{Hb}}, \theta_{\rho}$ , namely:

$$\begin{aligned}\theta_{\text{Hb}} &\sim N(40, 3^2) \\ \theta_{\rho} &\sim N_{\text{trunc}}(4, 1; \text{min} = 2, \text{max} = 8)\end{aligned}$$

where  $N_{\text{trunc}}$  is a truncated normal distribution with upper and lower values given by min and max. The variance hyperparameters  $\sigma_{\text{Hb}}^2$  and  $\sigma_{\rho}^2$  are given flat priors. Figure 1 shows the posterior distributions for all the parameters and hyperparameters of the model. The population level parameters are given the following informative priors:

$$\begin{aligned}d &\sim \text{Beta}(2, 38) \\ \text{Hb}_{50}^{\rho} &\sim N(30, 2^2) \\ \text{Hb}_{50}^R &\sim N(30, 2^2) \\ k &\sim N_{\text{trunc}}(10, 2^2; \text{min} = 0, \text{max} = 20)\end{aligned}$$

Convergence of the Metropolis Hastings algorithm was done by running 4 independent chains and computing the Gelman-Rubin statistic.



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**Appendix 1 Figure 1.** Posterior distributions of model parameters and hyperparameters.

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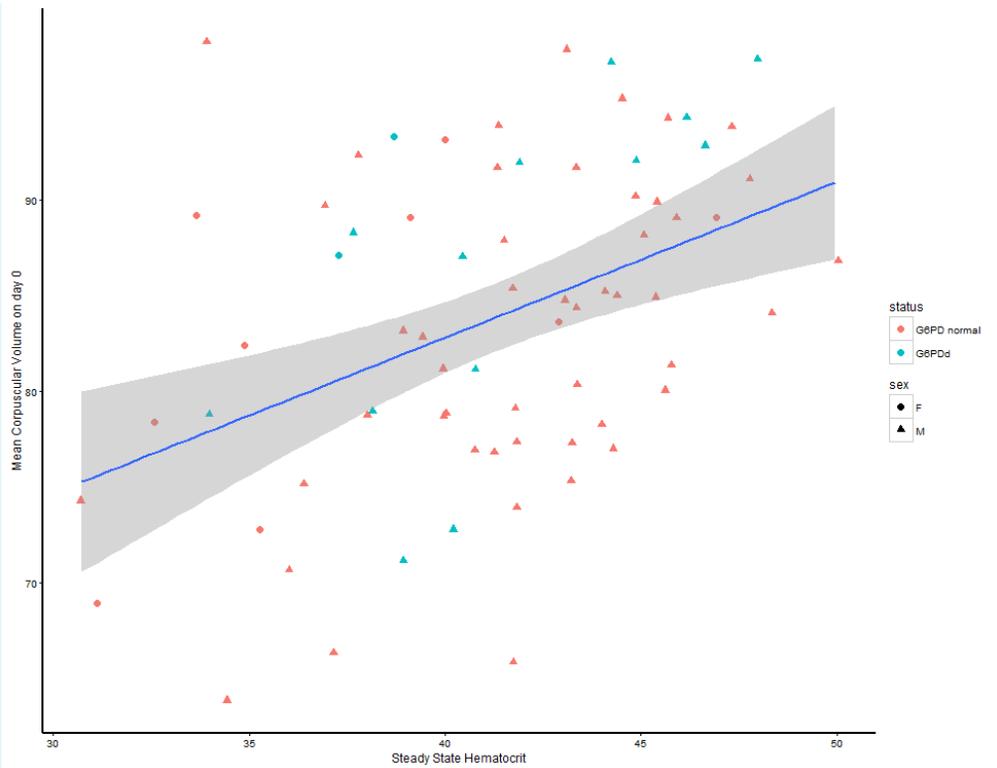
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As an independent check for model fit, Figure 2, compares the estimated individual steady state haematocrits with the data on individual mean corpuscular volume (MCV) on day 0. The relationship between these fitted estimates and the data from the study is approximately linear for all patients (G6PDd and G6PD normal). The MCV is a poor marker of anaemia; high values are associated with reticulocytosis, folate and B12 deficiency and low values with iron deficiency, and thalassaemia both of which are very common in tropical areas. A correlation with steady state haematocrit would suggest that the model is indeed estimating the correct quantities. Therefore if the model estimates of the steady state haematocrit are correct, this should in theory correlate well with the baseline MCV and suggest the model is converging to the correct quantities.



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**Appendix 1 Figure 2.** Relationship between the steady state haematocrit and the mean corpuscular volume at day zero. G6PDd patients are colored in blue, G6PD normal patients in red; women are shown by circles, men by triangles.

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### Sensitivity analysis

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Figure 3 shows the effect of the individual parameters of the model (except the killing parameter  $d$ , the main parameter of interest in the model) on the haematocrit and reticulocyte response when all other parameters are held fixed.

To illustrate the individual effects of the parameters, we take as example a daily dosing regimen with identical dose (i.e. same value of the parameter  $d$ ) for 30 days. This is chosen to approximate the drop in haemoglobin observed in G6PDd African  $A^-$  studies (Figure 1). We fix  $d = 0.05$ .

Some notable points:

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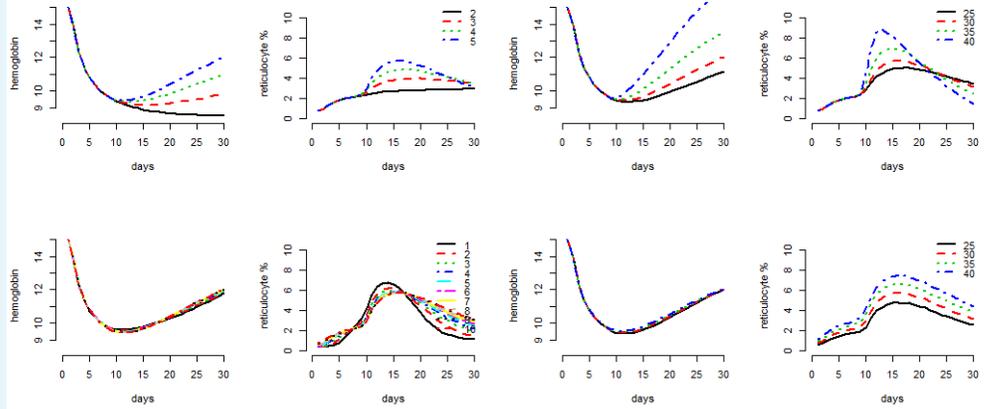
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- The max-fold increase ( $\rho^{\max}$ ) of RBCs in the bone marrow has the most impact on the reticulocyte response after day 9. We assume that a twofold increase in production is the minimum viable response to anaemia (shown by the red lines). *Hillman* estimates the max-fold increase in healthy males as approximately 5-fold.
- The analysis of the data from *Kheng et al. (2015)* gives very similar values for the population distribution of  $Hb_{50}^{\rho}$  as that estimated by *Hillman (1969)* (see Figure 1 in *Hillman (1969)*).



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**Appendix 1 Figure 3.** Individual parameter effects on the behavior of the compartmental model as shown by the haematocrit response and the reticulocyte count response. From top left to bottom right, grouped by pairs: the mid-haemoglobin concentration parameter  $Hb_{50}^R$  for the reticulocyte release function; the mid-haemoglobin concentration parameter  $Hb_{50}^\rho$  for the marrow production function; the hill coefficient  $k$  for the reticulocyte release function; the max-fold production factor  $\rho^{\max}$ . The different values plotted for each parameter are shown in the legend for reticulocyte response plot.