Title: Evaluating artesunate-amodiaquine deployment, efficacy and safety: an *in silico* pharmacological model.

Ki Bae Hong^{1 †‡}

Email: kibaehong78@gmail.com

Ian Hastings¹

Email: Ian.Hastings@lstm.ac.uk

Katherine Kay^{1 §*}

Email: KKay2@buffalo.edu

Eva Maria Hodel^{1*}

Email: EvaMaria.Hodel@lstm.ac.uk

* Equal contribution

‡ Corresponding author

¹ Liverpool School of Tropical Medicine, Liverpool, United Kingdom

[†] Current affiliation: Sindosi Ejin Pediatrics Hospital, Jangjae-ri 1789, Baebang-eup, Asan-si, Chungcheongnam-do, Republic of Korea

[§] Current affiliation: University at Buffalo State University of New York, Buffalo, New York, USA

Abstract

Background: The World Health Organization currently recommends artesunate-amodiaquine (AS-AQ) as a first-line treatment for uncomplicated falciparum malaria. The clinical efficacy of AS-AQ is very high but its effectiveness in the field varies considerably. This study aimed at comparing the efficacy, effectiveness and safety of AS-AQ fixed dose combination (FDC) and non-fixed formulation (non-FDC) in controlled and real-life settings using a pharmacological model of antimalarial treatment.

Methods: The effectiveness and safety of different drug formulations in different treatment scenarios were investigated using a pharmacological model of AS-AQ treatment. The model simulated multiple treatment scenarios to assess the effects of age- or weight-based dosing bands in three geographically distinct patient populations, and poor patient adherence.

Results: The model output was consistent with clinical trials in terms of cure rates, recrudescence rates and the pattern of AQ overdosing with age- and weight-based dosing regimens. AS-AQ treatment has good efficacy and effectiveness in fully adherent patients but monotherapy of AS or AQ lead to treatment failure. The weight-based dosing regimen with FDC was the best option for patients in terms of drug safety and had similar efficacies to the other regimens. Asians were more likely to be overdosed with AQ when using age-based dosing regimens.

Conclusions: Weight-based dosing is optimal but not always feasible, so age-based dosing regimens are often used as an alternative. The model outputs highlight the importance of optimising these age-based dosing regimens for specific regions, and identify an increased risk of overdosing in young children.

Max. word count 350; current 245

Keywords: Malaria, falciparum, antimalarials, amodiaquine, artemisinin, computer simulation, treatment outcome, patient compliance, pharmacology, pharmacokinetics

Introduction

The World Health Organization (WHO) estimated there were approximately 198 million cases of malaria and 584,000 deaths in 2013 (1). Due to the emergence of chloroquine (CQ), mefloquine (MQ) and sulphadoxine-pyrimethamine (SP) resistance in malaria endemic regions, treatment policy from WHO has been recommending artemisinin-based combination therapy (ACT) as a first-line treatment for uncomplicated *Plasmodium falciparum* malaria for almost a decade (2). Artemisinin derivatives are very potent but rapidly eliminated from the body and should therefore not be administered as monotherapies but in combination with a slower acting partner drugs able to sustain sufficient parasitocidal concentrations to clear all remaining parasites (2). Amodiaquine (AQ) is often used as a seasonal malaria chemoprevention together with SP in children less than five years old who live in high seasonal malaria transmission countries (3). Artesunate-amodiaquine (AS-AQ) is one of the ACTs recommended by the WHO and was adopted as the first-line treatment in West Africa approximately ten years ago (4-7). Recently, AS-AQ was also recommended as the first-line treatment for mass drug administration (MDA) in Ebola-affected countries (8, 9).

68
69 Studies of the WHO's three-day AS-AQ regimen found it to be highly efficacious and safe in the
70 treatment of uncomplicated falciparum malaria (10-16). However, while the clinical efficacy of
71 AS-AQ is high, its real-life effectiveness varies substantially; studies in Africa have shown AS-
72 AQ effectiveness to be between 63-85% (17-22). Many factors affect drug effectiveness in the
73 field, including poor access to treatment, provider compliance to treatment guidelines, or
74 adherence of patients and caregivers to prescriptions (23). AS-AQ can be prescribed as either
75 fixed-dose combination (FDC) formulations or non-FDC (i.e. loose tablets or co-blistered
76 tablets). FDCs include both AS and AQ in a single table, each containing either 25/67.5, 50/135
77 or 100/270 mg AS/AQ depending on tablet strength (24). While non-FDC co-blister packs
78 contain 50 mg of AS and 153 mg of AQ (25). Non-FDC formulations tend to be less user-
79 friendly and allow patients to take only one of the two ACT components. FDC formulations
80 reduce this problem and are recommended to increase adherence, thus delaying the development
81 of resistance to both drugs (26-29).

82
83 It is recommended that the dose of AS-AQ is calculated according to a patient's body weight
84 (30) but this can be logistically challenging in developing countries. Some health facilities for
85 example may not have functional scales (10, 31). In practice, the amount of drug given to
86 patients is commonly based on their age, which can lead to inaccurate dosing.

87
88 Clinical trials are mainly designed to test safety and efficacy of interventions, so the results of
89 trials provide guidance for diagnosis, treatment and prevention. However, conducting clinical
90 trials is expensive, time-consuming and restricted by ethical constraints, particularly when their

aim is to directly investigate the impact of poor patient adherence. Pharmacological models of drug treatment can overcome these problems and allow researchers to investigate drug effectiveness and drug regimen safety. Historically, pharmacological models of antimalarial drug treatment focused on monotherapies (32-37) but since the WHO recommendation of ACTs, the model methodologies have been extended to include combination therapies (38-44). This study used a pharmacological model for AS-AQ treatment to compare the efficacy, effectiveness and safety of different dosing regimens and FDC versus non-FDC in controlled and real-life settings. Moreover, this study evaluated the change of drug efficacy and effectiveness when patients adhere poorly to the recommended regimen.

Methods

Pharmacological model

This study adapted the pharmacological model described in Kay and Hastings (41) for the simulation of AS-AQ treatment outcome. The model, implemented in the statistical software R (version 3.1.0), tracks the number of parasites P over time t after treatment using the following differential equation (Equation 10 in (41))

$$\frac{dP}{dt} = P \left(a - f(I) - \sum_{d=1}^r f(C_d) \right) \quad (1)$$

where P is the number of parasites, a is the parasite growth rate, $f(I)$ is the host's background immunity to the infection (which is assumed to kill parasites or slow their growth), r is the number of drugs and/or their active metabolites in the regimen and $f(C_d)$ describes the drug effect (i.e. its kill rate) for each drug d depending on its concentration C_d (mg/L). Integrating Equation 1 gives the number of parasites P at time t (Equation 16 in (41))

$$P_t = P_0 e^{(a-f(I))t} \prod_{d=1}^r e^{-\int_0^t f(C_d) dt} \quad (2)$$

where P_t is the number of parasites at time t , P_0 is the number of parasites at the start of treatment. In this study, the hosts' background immunity was ignored and $f(I)$ was set to zero so that all patients were malaria naive and had no acquired immunity.

The drug-dependent killing function for each drug d is described by the standard Michaelis-Menton equation, i.e.

$$f(C) = V_{max} \left(\frac{C^n}{C^n + IC_{50}^n} \right) \quad (3)$$

where V_{max} is the maximal drug-killing rate, n is the slope of the dose response curve, and IC_{50} is the concentration at which 50% of the maximal killing rate occurs.

For AS and its active metabolite dihydroartemisinin (DHA), the model tracked drug concentration over time using a standard one-compartment disposition model allowing for the absorption of the parent drug (i.e. AS) across the gut wall at rate k_a and conversion to its active metabolite DHA at rate k_m (**Figure 1**). Equations for tracking drug concentration over time are described in the original model by Kay and Hastings (41).

For AQ and its active metabolite desethylamodiaquine (DEAQ), pharmacokinetics follow a more complex model. AQ and DEAQ were modelled as two separate drugs using a pair of two-compartment disposition models and a shared estimate of the absorption rate constant, k_a (**Figure 2**); this approach was proposed and validated by Hietala et al (45). The model used the equation describing a drug with a three-compartment disposition (Equation 1.72 in (46)) but simplified to simulate two-compartments by setting the inter-compartmental clearance between compartments 1 (the central, blood compartment) and 3 (the unused peripheral compartment) to zero. The model equations assume the drug has first-order absorption, linear elimination and allows for multiple doses without lag time so that the amount of drug C present in the central compartment at time t is

$$C(t) = \sum_{i=1}^n D_i \left(A e^{-\alpha(t-t_{Di})} + B e^{-\beta(t-t_{Di})} \right) + C e^{-\gamma(t-t_{Di})} - (A + B + C) e^{-k_a(t-t_{Di})} \quad (4)$$

where D is amount of drug (in mg) given in the i^{th} dose. A , B , C are macro-constants and α , β and γ are rate constants (see (46) for more details).

Both drugs in the AS-AQ combination have two active forms, an active parent drug and an active metabolite. The model therefore determines four drug concentrations and four corresponding estimates of drug dependent killing $f(C)$ at each time step. The drug killing of the parent drug and its metabolite were assumed to have similar modes of action and so their effect cannot be additive (as implied in Equation 1). So at the end of each time step, only the drug form with the higher parasite-killing rate contributed to drug effect. The AS/DHA and AQ/DEAQ forms with the higher parasite-killing rate were combined assuming additive drug action to update parasite numbers (for more details see methods of (41)).

To simulate dosing according to age or weight, region-specific weight-for-age references for a global population and African, Asian, and Latin American populations were included as described previously (47). In brief, each individual was randomly assigned an age between six months to 25 years and their weight was read from the regional references (48) for a randomly selected weight-for-age percentile.

Calibration and validation

Pharmacokinetic (PK) and pharmacodynamic (PD) parameters of AS and its metabolite DHA were previously validated in (41). The PK/PD parameters for AQ and DEAQ and their coefficient of variation (CV) estimates were extracted from the literature and are summarized in **Table 1**. For parameters where no CV was provided in the literature a default value of 30% was selected (as was done previously e.g. (40, 41)). It was assumed that parameters were normally distributed if the CV is $\leq 50\%$ and log-normally distributed if they were $> 50\%$.

The PK parameters were validated by comparing the simulated maximal plasma concentration (C_{max}) and time to C_{max} (T_{max}) of AQ and DEAQ to field observations (45). The PD parameters (40) were validated by matching simulated cure rates, parasite clearance times (PCT) and periods of chemoprophylaxis (PoC) to those estimated in clinical trials. The PCT is the time taken for the infection to fall below the limit of microscopic detection (defined here as $<10^8$ parasites (49)). The PoC measures the time until new infections can occur after treatment, which is the duration of time that a drug suppresses the new infection. The PD parameters were taken from (50-53) and adjusted until the simulated cure rates, PCT and PoC matched field observations of the 28-day cure rate 96-98% (data from Nigerian children and Indian adults and children (13, 54) who were fully adherent and received age- or weight-based FDC regimens), the PCTs in these datasets were 1 ± 0.6 days (54-56) and PoC were 22 – 25 days (57). These results were obtained assuming full adherence to an age-based FDC or non-FDC regimens from clinical trials usually enrolled children from Africa. The final PK and PD parameters are given in Table 1 and are well within the range observed in the clinic and laboratory (41, 45, 50-53, 58).

Simulation and analysis

This study compared the effectiveness and safety of different treatment regimens and drug formulations for the adherence scenarios listed in **Table 2**. Weight-based dosing regimens with fixed-dose and co-blistered combinations of AS-AQ were based on drug dose per patients' weight band as recommended by WHO ((2); **Table 3**). Note, individuals under 5 kg were excluded from this analysis and replaced by a new random sample record. Age-based dosing

regimens were selected from clinical trials (11, 59, 60) as WHO recommends only weight-based dosing regimens. The age- and weight-based regimens used in this study are presented in **Table 3**.

The details of the different model runs are outlined in **Table 4**. A single model ‘run’ simulates 10,000 individuals for each of the regional weight-for-age distributions (representative of either a global, African, Asia or Latin American populations) and followed patients for 28 days after the start of treatment (i.e. the recommended follow-up duration according to the WHO guidelines (26)). Treatment outcome at 28 days was defined as follows; 1) all parasites cleared ($P_t < 1$); 2) parasite are still present but below limit of microscopic detection (LoD) of $P_t < 10^8$ and might subsequently either clear or recrudesce; 3) recrudescence which fell below LoD at some point during follow-up but later recrudesced to above LoD at some point before the end of the 28 day follow-up; 4) parasites are never cleared ($P_t > 10^8$) and are always detectable during entire post-treatment period. Categories 1) and 2) were classified as clinical cures and categories 3) and 4) were classified as clinical failures (this corresponds to what is defined as cured/failures in the field).

To reproduce results of drug effectiveness in a real life setting, the model accounted for missed doses and poor adherence, using patterns of non-adherence found in the literature (61, 62). An adherence level defined the proportion of patients that followed the recommended treatment regimens; seven adherence levels were investigated here i.e. 50%, 60%, 77%, 80%, 85%, 90% and 100%. So an adherence level of 77% for example, means 77% of patients followed the full

three day course of AS-AQ treatment and 23% followed one of the alternative scenarios listed in **Table 2**.

Safety was assessed by determining the proportion of patients who received an AS and AQ dose within the therapeutic range. The therapeutic range of AS was defined as 2–10 mg/kg/day and 7.5–15 mg/kg/day for AQ (2). The magnitude of under- and overdosing of AS and AQ was determined as well as median values of C_{max} of AS, DHA, AQ, and DEAQ.

Results

Efficacy and effectiveness of the WHO recommended regimen

Efficacy describes how a drug performs under ideal conditions for example, when treatment is directly observed in clinical trials. In contrast, effectiveness describes how well a drug works in a real-life setting where, for instance, patients take medication unsupervised. The efficacy of the WHO recommended target dose of 4 mg/kg AS and 10 mg/kg AQ daily (regimen A) with full-adherence (scenario 1) was compared with the effectiveness of the same regimen in patients with varying degrees of non-adherence (scenarios 2–6) at day 28 of follow-up (**Table 4** and **Figure 3**). Scenario 1 assumed all individuals took the full three days of treatment and resulted in a cure rate of approximately 96%. When the third dose was missed (scenario 2) cure rates decreased to 80–94% depending on the proportion of non-adherent patients (**Figure 3A**). However, the WHO regimen appears relatively robust with clinical cure rates remaining above 90% when half the patient population only takes the first two treatment doses. In contrast, when 10% of patients

omitted both the second and third doses (scenario 3) cure rates dropped to 89% (**Figure 3B**). The proportion of patients cured drops to 23% when only the first dose of treatment is taken. Delaying the second dose by 12 hours (scenario 4) did not alter the drug effectiveness. Monotherapies of either AQ or AS given for three days (scenarios 5 and 6 respectively) showed low cure rates of 36% and 23% respectively (**Table 4**).

In order to measure the immediate therapeutic response to the drugs, PCT was calculated. In scenarios 1 and 4, the median PCT was 1.25 days and the median time to recrudescence was 22 days (data pooled over all the regimens) in both cases (**Figure 4**). The median PCT did not change until patients missed more than one dose of the combination. As expected, monotherapies with the fast acting, short-lived component AS, had a negligible impact on the median PCT but shortened the median time to recrudescence. Conversely, monotherapies with the slower acting, longer-lived AQ partner drug lengthened the median PCT but did not affect the time to recrudescence.

Efficacy and effectiveness

The cure rates of the six simulated age- and weight-based regimens (B to G, **Table 3**) were all above 96% when patients were fully adherent to the three day regimen (scenario 1, **Table 4**). Cure rates in the three regions were compared for each regimen and, were highest in Asia (97%) and lowest in Latin America (95%) assuming complete adherence to the regimen (**Figure 5A**). When all patients followed scenario 2 (i.e. omitted the third doses), the cure rates dropped below 90% in all six regimens (**Figure 5B**) and when all patients followed scenario 3 (i.e. omitted the

second and third doses), the cure rates dropped to below 35% (**Figure 5C**). These results emphasize that full three day course of AS-AQ is necessary to treat uncomplicated falciparum malaria.

Drug dosing and safety evaluation

In scenario 1, all regimens showed high cure rates (i.e. >95 %) but selection of the ‘best’ or optimal regimen should also depend on the regimen’s safety profile. In absence of an absolute ‘toxic’ mg/kg dose or plasma concentration threshold, relatively ‘safe treatments’ were determined in the simulations using the proportion of patients who received doses in the recommended therapeutic range of AS and AQ. The therapeutic range was defined as 2–10 mg/kg/day for AS and 7.5–15 mg/kg/day for AQ (26). **Table 5** and **Figure 6** show the proportion of patients under- or overdosed with either AS or AQ for each of the regimens described in **Table 3**. In the simulated populations 15% of patients received doses above and 4.4% below the therapeutic dose range of AQ. This was an average across all regimens and regions but there was considerable differences between regimens. For example, no patients following Regimen B (in all three continents) were overdosed. In addition, the two age-based dosing regimens seems more likely to over- or under-dose AQ. Asians are more likely to be overdosed and Latin Americans more likely to be under dose than any other regions (**Figure 6**).

The specific dose of AQ each patient received is given on **Figure 7** using examples of one age- and one or weight-based regimen (**Table 3**, regimens B and D respectively). The weight-based dosing regimen with FDC (regimen B) showed that overdosing of AQ was uncommon in all ages

(**Figure 7A**). The age-based dosing regimen showed that patients near the lower cutoff of each age band tended to receive higher mg/kg dosages and so were more frequently overdosed with AQ (**Figure 7B**). The proportion of patients overdosed decreased with increasing patient age with a small proportion of patients at the upper dosing band cut-offs being under-dosed (**Figure 7B**).

Discussion

This study adapted an existing antimalarial pharmacological model to simulate AS-AQ treatment and compare the efficacy, effectiveness and safety of a variety of AS-AQ regimens based on fixed dose and non-fixed formulations and using either age- or weight-based dosing bands. The structural model used herein for AQ and its active metabolite was described previously (45), but the literature provides several alternative structural PK models (45, 63, 64). AQ and DEAQ were modelled as separate drugs with two-compartment PK model structures because it can be assumed that AQ and DEAQ have similar physiochemical characteristics such as their absorption, distribution, solubility and stability. Furthermore, the separate two-compartment PK models have an algebraic solution (41, 46) that makes it computationally preferable to a linked four-compartment PK model. Recently, more sophisticated PK models have become available using non-linear mixed-effect modeling (65) and our analysis could be extended to incorporate these more complex dynamics. In the meantime, we note that the simpler models provide a good fit to PK data and therefore constitute a good platform for our primary objectives of investigating the effects of age- and weight-based dosing bands and the impact of poor patient adherence.

The literature used to calibrate the PK model for AQ reported large inter-individual variation of PK parameters (45, 66), i.e. for absorption rate the CV was 100% and for volume of distribution the CV was 139%. Despite this, those CVs originating from a study that enrolled children were retained because they came from the study with the largest sample size. The CVs for the other PK parameters, i.e. k_a , CL , Vd_c , were too narrow and resulted in model output that was inconsistent with field data (45, 53). Consequently, CVs from a separate study that enrolled women with *P. vivax* malaria during and after pregnancy were used (58). Although, the volume of distribution in pregnant women was larger than in non-pregnant women, the variation of the PK parameters in that study was smaller than the one involving children. Where no CV for AQ/DEAQ specific PK and PD parameters could be found in the literature, the CV was set to 0.3 (for further discussion see (41)). Note that the model ignored the acquired immunity of malaria as there is currently no consensus mathematical description of immune acquisition (see for example, (40, 67-69)) but it is important that the model can predict efficacy and effectiveness in the most vulnerable, i.e. non-immune, individuals. The cure rate for validation was thus selected from studies conducted in Asia or Africa (the latter with pediatric patients) because these populations are presumed to have relatively low immunity to falciparum malaria. Overall, the pharmacological model used here was able reproduce field data (13, 45, 54, 56, 70).

The model generated cure rates, recrudescence rates and the safety profiles for six different regimens. The efficacies of all six regimens were high when patients adhered to the full three-day course of AS-AQ (scenario 1 and 4) and, as expected, lower when patients did not follow the three-day course (scenario 2 and 3). Monotherapies of either drug taken for three days (scenario 5 and 6) also showed low cure rates as reported from clinical trials comparing monotherapy to

combination therapy (13, 55). This study also investigated safety of different dosing regimens and combinations. The weight-based dosing with FDC (regimen B) was the best option in regard to safety and was similar in terms of efficacy compared to the other regimens. Over- and under-dosing of AQ was a significant problem in the age-based dosing regimens.

The pharmacological model generated similar proportions of cure rates with those observed in clinical trials for AS-AQ three day course treatments. Many clinical trials reported polymerase chain reaction (PCR)-corrected cure rates of 97% or above at day 28 or 42 after treatment when patients were treated with 10mg/kg of AQ for three days combined with artesunate (11, 13, 60, 71). The model also generated 97% cure rate for full adherence. However, the model cure rates following AQ monotherapy (10mg/kg once daily for three days) were just 36% if cure rate is defined as patients who clear all parasites or 68% if cure rate is defined as patients who had parasites below the limit of microscopic detection. This is much lower than the day 28 PCR-corrected cure rates from equivalent clinical trials using AQ monotherapy, i.e. 88% in India (13), 54% in Kenya (72), 79% in Senegal (72) and 85% in Gabon (72). In addition, when model follow-up to 28 days, the simulated cure rates dropped to 36% (defined as the proportion of patients with undetectable parasites). The IC_{50} values for AQ and DEAQ in this study might reflect resistant strains, thereby generating a lower cure rate than clinical trials. When IC_{50} was decreased from 0.02 mg/L to 0.01 mg/L, model cure rates become more similar to those reported in the studies i.e. 54% of patients cleared all parasites and 84 % of patients had undetectable parasites. These result suggests that *P. falciparum* is likely to be sensitive to AQ in Gabon but not in Kenya(72-74); possible reflecting the much higher usage of AQ in West- compared to East-Africa. As expected, these results indicate that monotherapy had risks of treatment failure

and emergence of drug resistance. Therefore, intensive effort is required to reduce monotherapy treatment by improving patients' adherence.

Adherence to ACT varies across regions, ranging from 48-94% (23, 75). To increase patient adherence, AS-AQ treatment is now available in co-blistered packaging or as FDC and the reported efficacies are more than 95% (29, 60). The Worldwide Antimalarial Resistance Network (WWARN) recently conducted a pooled analysis that included 43 studies with 9,106 patients treated with a three day course of AS-AQ for uncomplicated falciparum malaria (7). Their study compared the efficacy of several AS-AQ combinations and found the efficacy of FDC (98.1%) and co-blistered non-FDC (97.9%) were similarly high but found the efficacy of loose non-FDC-30 (95%) was statistically significantly lower (7). The median AQ dosed received in the WWARN's pooled analysis was 32.4 mg/kg for FDC (AS/AQ ratio 2.7) and 35.3 mg/kg for co-blister non-FDC (AS/AQ ratio 3.06) (7), which was very similar to that used in this model, i.e. a median AQ dose of 32.8 mg/kg for FDC and 35.9 mg/kg in co-blister non-FDC, and resulted in very similar AS/AQ ratios. It is gratifying to note the simulated cure rates for AS-AQ treatment with FDC and co-blistered non-FDC were highly consistent to the findings from the WWARN study. Patients in both the WWARN pooled analysis and the simulation receiving non-FDC formulations, tended to have slightly higher median dose of AQ. The proportion of patients receiving AQ doses below the therapeutic dose range was similarly consistent at 3.4% and 4.4% in the analysis by the WWARN AS-AQ study group and the simulation respectively. The only small deviation between the results of WWARN and the simulated patients occurred when measuring the proportion of patients under-dosed. WWARN report under-dosing with FDC in 1.1% of patients and with co-blister non-FDC in 0.9% (7) of patients while the simulation

predicted under-dosing in 5.1% and 3.8% respectively. Since FDC contains less amount of AQ than co-blister combination, FDC had higher risk of under-dosing. However, the difference was small. Under-dosing has the potential to affect efficacy and effectiveness and the model predicted results highly consistent with those presented in WWARN's pooled analysis (7) except when simulating the proportion of patients receiving sub-therapeutic AQ doses.

Patients overdosed with AQ are more prone to vomiting than those receiving the appropriate dose (76), a side effect that can make patients and caregivers reluctant to administer treatment (61). When treating children with AS-AQ, overdosing of the AQ component is a particular concern regardless of whether children are dosed according to their age or weight. A study in Senegal (10) gave patients co-blistered AS-AQ (AS 50 mg, AQ 153 mg) based on weight or age; 2.1% of patients were overdosed with AQ when dosed according to weight and 17.8% were overdosed with AQ when dosed according to age. WWARN's pooled AS-AQ analysis (7) showed children received the lowest AQ total dose if they were under 12 months old (28.9 mg/kg FDC vs 32.6 mg/kg co-blistered non-FDC) and the highest AQ per dose if they were 1–4 years old (33.8 mg/kg FDC vs 38.3 mg/kg co-blistered non-FDC) (7). The simulated results presented here show similar patterns of under-dosing: Patients less than 12 months old received 28.2 mg/kg with FDC and 32.0 mg/kg with non-FDC and children aged 1–4 years old received 35.7 mg/kg with FDC and 40.5 mg/kg with non-FDC. Non-FDC formulations also tended to result in slightly higher amounts of AQ in all age groups and this trend was similar in the WWARN study (7). The authors of the WWARN study credited higher failure rates for the non-FDC products to the fact that the FDC product “was developed using a weight-for-age reference database from malaria endemic countries, to ensure optimal dosing with the pediatric

formulation”. When “applied either by weight- or age-based criteria” the FDC product “probably increases dosing accuracy, and the availability of different tablet strengths, including a pediatric formulation, obviates the need for tablet splitting, reduces the pill burden and potentially improves adherence”

The WHO recommends that first-line treatment should be changed when failure rates exceed 10% (2). In this study, the clinical failure rate was less than 5% (assuming full adherence for all regimens) indicating that current treatment policies are adequate. Clinical failure rates increased dramatically to 15% when patients missed one dose of AS-AQ. As per WHO recommendations failure rate of 15% are expected to result in a change of treatment regimen. However, the importance of ensuring patient adherence with recommended drug doses and schedules was also simulated. Non-adherence was incorporated in the models and used to show that cure rates could be improved to above 90% if less than half the simulated population missed one dose of AS-AQ combination treatment or if no more than 10% of the population missed two doses of AS-AQ. Clearly careful monitoring of patient adherence is needed for successful control of malaria but is difficult to quantify in practice (23). Non-adherence often leads to more patients with sub-therapeutic drug levels. This causes two main problems, first more patients fail treatment and often require re-treating which in turn results in higher treatment costs and second, sub-therapeutic drug levels may increase the appearance and subsequent spread of drug resistance.

The WHO guidelines (2) recommend that treatment doses be calculated according to the patients’ body weight (30). In most of the malaria endemic countries, dosing by age is a more common practice, in some cases due to the lack of functional scales (77) but more often because

health workers prefer to prescribe according to age for simplicity (31). This study showed that simulated patients who received age-based doses had highest AQ levels in the younger ages within each age band (not surprising given that younger patients tend to be lighter). A clinical trial in Senegal showed that dosing patients according to body weight resulted in more correct AQ doses; 18% of patients were overdosed with AQ when treated according to age and 13% were overdosed with AQ when treated according to body weight (10). These results show the benefits to patients that result from the use of regional weight-for-age distributions to optimize age-based dosing regimens (78).

When comparing the results across different populations globally, these simulated results show Asian populations are at the highest risk of receiving AQ above the therapeutic range and Latin American populations had the highest risk of receiving AQ below the therapeutic range. This become particularly apparent when simulated patients were dosed according to their age. One possible explanation is that Asian people tend to weigh less than Latin Americans (48). This reinforces the importance of using regional weight-for-age distributions to inform treatment decisions at a regional or country level.

Conclusion

In conclusion, the simulated probability of receiving an AQ over-dose and predicted drug effectiveness were highly consistent with clinical trials. Efficacy and effectiveness were high in all regimens when simulating fully adherent patients, but FDCs have a major advantage in ensure patients take both dugs in the ACT so minimizing the risks associated with patients taking AS or

AQ monotherapies. The FDC weight-based dosing regimens had better proportion of patients receiving the recommended target dose and will remain the preferred regimen. However, it must be recognized that this is not always feasible, whether it is due to technical difficulties locally, healthcare preference or simply a logistical problem, for example in large MDA campaigns. Age-based dosing regimens should therefore be designed with consideration of the region-specific the probability of AQ over-dosing to reduce the risk of overdosing in the most at-risk populations.

460 **Abbreviations**

461	ACT	artemisinin-based combination therapy
462	AQ	amodiaquine
463	AS-AQ	artesunate-amodiaquine
464	CQ	chloroquine
465	CV	coefficient of variation
466	DEAQ	desethylamodiaquine
467	DHA	dihydroartemisinin
468	FDC	fixed-dose combination
469	LoD	limit of microscopic detection
470	MDA	mass drug administration
471	MQ	mefloquine
472	PCR	polymerase chain reaction
473	PCT	parasite clearance times
474	PD	pharmacodynamic
475	PK	pharmacokinetic
476	PoC	periods of chemoprophylaxis
477	SP	sulphadoxine-pyrimethamine
478	WHO	World Health Organization
479	WWARN	Worldwide Antimalarial Resistance Network

480

481 **Ethics approval and consent to participate**

482 Not applicable

483

484 **Consent for publication**

485 Not applicable

486

487 **Competing interests**

488 The authors declare that they have no competing interests

489

490

491 **Funding**

492 This work was funded by the Bill and Melinda Gates Foundation (grant 37999.01) and the

493 Medical Research Council (grant G110052) and supported by the Liverpool School of Tropical

494 Medicine.

495

496 **Authors' Contributions**

497 EMH & KK conceived the study. KBH calibrated and validated the model, and generated the

498 results. All authors interpreted the results. KBH wrote the first draft of the manuscript. All

499 authors gave critical input and contributed to the writing of the manuscript. All authors have read

500 and approved the final manuscript.

501

Reference

1. WHO. World Malaria Report 2014. Geneva: World Health Organization; 2014.
2. WHO. Guidelines for the treatment of malaria. Geneva: World Health Organization; 2015.
3. WHO. WHO Policy Recommendation: Seasonal Malaria Chemoprevention (SMC) for *Plasmodium falciparum* malaria control in highly seasonal transmission areas of the Sahel sub-region in Africa. 2012 March 2012.
4. Kremsner PG, Krishna S. 2004. Antimalarial combinations. Lancet. doi:10.1016/s0140-6736(04)16680-4
5. Kerb R, Fux R, Morike K, Kremsner PG, Gil JP, Gleiter CH, et al. 2009. Pharmacogenetics of antimalarial drugs: effect on metabolism and transport. Lancet Infect Dis. doi:10.1016/s1473-3099(09)70320-2
6. Oguche S, Okafor HU, Watila I, Meremikwu M, Agomo P, Ogala W, et al. 2014. Efficacy of artemisinin-based combination treatments of uncomplicated falciparum malaria in under-five-year-old Nigerian children. The American journal of tropical medicine and hygiene. doi:10.4269/ajtmh.13-0248
7. Group TWWARNA-AS. 2015. The effect of dosing strategies on the therapeutic efficacy of artesunate-amodiaquine for uncomplicated malaria: a meta-analysis of individual patient data. BMC Med. doi:10.1186/s12916-015-0301-z
8. WHO. Guidance on temporary malaria control measures in Ebola-affected countries. Geneva, Switzerland: World Health Organization; 2014.
9. WHO. Malaria: Preventive therapies: World Health Organization; 2015 [Available from: http://www.who.int/malaria/areas/preventive_therapies/en/].

- 525 10. Brasseur P, Agnamey P, Gaye O, Cisse M, Badiane M, Vaillant M, et al. 2009. Dosing
526 accuracy of artesunate and amodiaquine as treatment for falciparum malaria in Casamance,
527 Senegal. *Tropical medicine & international health : TM & IH*. doi:10.1111/j.1365-
528 3156.2008.02190.x
- 529 11. Espie E, Lima A, Atua B, Dhorda M, Flevaud L, Sompwe EM, et al. 2012. Efficacy of
530 fixed-dose combination artesunate-amodiaquine versus artemether-lumefantrine for
531 uncomplicated childhood Plasmodium falciparum malaria in Democratic Republic of Congo: a
532 randomized non-inferiority trial. *Malaria journal*. doi:10.1186/1475-2875-11-174
- 533 12. Faye B, Offianan AT, Ndiaye JL, Tine RC, Toure W, Djoman K, et al. 2010. Efficacy
534 and tolerability of artesunate-amodiaquine (Camoquin plus) versus artemether-lumefantrine
535 (Coartem) against uncomplicated Plasmodium falciparum malaria: multisite trial in Senegal and
536 Ivory Coast. *Tropical medicine & international health : TM & IH*. doi:10.1111/j.1365-
537 3156.2010.02487.x
- 538 13. Anvikar AR, Sharma B, Shahi BH, Tyagi PK, Bose TK, Sharma SK, et al. 2012.
539 Artesunate-amodiaquine fixed dose combination for the treatment of Plasmodium falciparum
540 malaria in India. *Malaria journal*. doi:10.1186/1475-2875-11-97
- 541 14. van den Broek I, Kitz C, Al Attas S, Libama F, Balasegaram M, Guthmann JP. 2006.
542 Efficacy of three artemisinin combination therapies for the treatment of uncomplicated
543 Plasmodium falciparum malaria in the Republic of Congo. *Malaria journal*. doi:10.1186/1475-
544 2875-5-113
- 545 15. Thanh NX, Trung TN, Phong NC, Quang HH, Dai B, Shanks GD, et al. 2012. The
546 efficacy and tolerability of artemisinin-piperaquine (Artequick(R)) versus artesunate-

- amodiaquine (Coarsucam) for the treatment of uncomplicated *Plasmodium falciparum* malaria in south-central Vietnam. *Malaria journal*. doi:10.1186/1475-2875-11-217
16. Ndounga M, Mayengue PI, Casimiro PN, Loumouamou D, Basco LK, Ntouni F, et al. 2013. Artesunate-amodiaquine efficacy in Congolese children with acute uncomplicated *falciparum* malaria in Brazzaville. *Malaria journal*. doi:10.1186/1475-2875-12-53
17. Oyakhirome S, Potschke M, Schwarz NG, Dornemann J, Laengin M, Salazar CO, et al. 2007. Artesunate--amodiaquine combination therapy for *falciparum* malaria in young Gabonese children. *Malaria journal*. doi:10.1186/1475-2875-6-29
18. Pereira EA, Ishikawa EA, Fontes CJ. 2011. Adherence to *Plasmodium vivax* malaria treatment in the Brazilian Amazon Region. *Malaria journal*. doi:10.1186/1475-2875-10-355
19. Cohen JL, Yavuz E, Morris A, Arkedis J, Sabot O. 2012. Do patients adhere to over-the-counter artemisinin combination therapy for malaria? evidence from an intervention study in Uganda. *Malaria journal*. doi:10.1186/1475-2875-11-83
20. Mace KE, Mwandama D, Jafali J, Luka M, Filler SJ, Sande J, et al. 2011. Adherence to treatment with artemether-lumefantrine for uncomplicated malaria in rural Malawi. *Clinical infectious diseases* : an official publication of the Infectious Diseases Society of America. doi:10.1093/cid/cir498
21. Souares A, Lalou R, Sene I, Sow D, Le Hesran JY. 2009. Factors related to compliance to anti-malarial drug combination: example of amodiaquine/sulphadoxine-pyrimethamine among children in rural Senegal. *Malaria journal*. doi:10.1186/1475-2875-8-118
22. WHO. Summary of treatment failure rates among patients infected with *P. falciparum*, grouped by treatment and country. World Health Organization; 2015.

- 569 23. Banek K, Lalani M, Staedke SG, Chandramohan D. 2014. Adherence to artemisinin-
570 based combination therapy for the treatment of malaria: a systematic review of the evidence.
571 Malaria journal. doi:10.1186/1475-2875-13-7
- 572 24. winthrop MA. Name of The Medical Product. 2010.
- 573 25. USAID. Guidelines for Managing the Malaria Supply Chain. U.S. ; 2011.
- 574 26. WHO. Guidelines for the treatment of malaria. Geneva, Switzerland: World Health
575 Organization; 2010.
- 576 27. Ratsimbaoa A, Ravony H, Vonimpaisomihanta JA, Raherinjafy R, Jahevitra M,
577 Rapelanoro R, et al. 2012. Compliance, safety, and effectiveness of fixed-dose artesunate-
578 amodiaquine for presumptive treatment of non-severe malaria in the context of home
579 management of malaria in Madagascar. The American journal of tropical medicine and hygiene.
580 doi:10.4269/ajtmh.2012.11-0047
- 581 28. Faucher JF, Aubouy A, Adeothy A, Cottrell G, Doritchamou J, Gourmel B, et al. 2009.
582 Comparison of sulfadoxine-pyrimethamine, unsupervised artemether-lumefantrine, and
583 unsupervised artesunate-amodiaquine fixed-dose formulation for uncomplicated plasmodium
584 falciparum malaria in Benin: a randomized effectiveness noninferiority trial. J Infect Dis.
585 doi:10.1086/599378
- 586 29. Ndiaye JL, Randrianarivelosia M, Sagara I, Brasseur P, Ndiaye I, Faye B, et al. 2009.
587 Randomized, multicentre assessment of the efficacy and safety of ASAQ--a fixed-dose
588 artesunate-amodiaquine combination therapy in the treatment of uncomplicated Plasmodium
589 falciparum malaria. Malaria journal. doi:10.1186/1475-2875-8-125

30. WHO. Antimalarial drug combination therapy: report of a WHO Technical Consultation. Geneva: World Health Organization; 2001. Contract No.: Document no. WHO/CDS/RBM/2001.35.
31. Souares A, Lalou R, Senghor P, Le Hesran JY. 2010. Child age or weight: difficulties related to the prescription of the right dosage of antimalarial combinations to treat children in Senegal. Transactions of the Royal Society of Tropical Medicine and Hygiene. doi:10.1016/j.trstmh.2009.07.018
32. Austin DJ, White NJ, Anderson R. 1998. The dynamics of drug action on the within-host population growth of infectious agents: melding pharmacokinetics with pathogen population dynamics. J Theor Biol.
33. Hoshen MB, Na-Bangchang K, Stein WD, Ginsburg H. 2000. Mathematical modelling of the chemotherapy of *Plasmodium falciparum* malaria with artesunate: postulation of 'dormancy', a partial cytostatic effect of the drug, and its implication for treatment regimens. Parasitology.
34. Hoshen MB, Stein WD, Ginsburg H. 1998. Modelling the chloroquine chemotherapy of *falciparum* malaria: the value of spacing a split dose. Parasitology.
35. Hoshen MB, Stein WD, Ginsburg HD. 2001. Pharmacokinetic-pharmacodynamic modelling of the antimalarial activity of mefloquine. Parasitology.
36. Saralamba S, Pan-Ngum W, Maude RJ, Leea SJ, Tarning J, Lindegårdh N, et al. 2011. Intrahost modeling of artemisinin resistance in *Plasmodium falciparum*. PNAS.
37. Simpson JA, Jansen KM, Price RN, White NJ, Lindegårdh N, Tarning J, et al. 2009. Towards optimal design of anti-malarial pharmacokinetic studies. Malaria journal. doi:10.1186/1475-2875-8-189

38. Hoshen MB, Stein WD, Ginsburg H. 2002. Mathematical modelling of malaria chemotherapy: combining artesunate and mefloquine. *Parasitology*.
39. Zaloumis S, Humberstone A, Charman SA, Price RN, Moehrle J, Gamo-Benito J, et al. 2012. Assessing the utility of an anti-malarial pharmacokinetic-pharmacodynamic model for aiding drug clinical development. *Malar J*.
40. Winter K, Hastings IM. 2011. Development, evaluation, and application of an in silico model for antimalarial drug treatment and failure. *Antimicrobial agents and chemotherapy*. doi:10.1128/aac.01712-10
41. Kay K, Hastings IM. 2013. Improving pharmacokinetic-pharmacodynamic modeling to investigate anti-infective chemotherapy with application to the current generation of antimalarial drugs. *PLoS Comput Biol*. doi:10.1371/journal.pcbi.1003151
42. Kay K, Hodel EM, Hastings IM. 2014. Improving the role and contribution of pharmacokinetic analyses in antimalarial drug clinical trials. *Antimicrobial Agents and Chemotherapy*. doi:10.1128/aac.02777-14
43. Hodel EM, Kay K, Hayes D, Terlouw D, Hastings I. 2014. Optimizing the programmatic deployment of the anti-malarials artemether-lumefantrine and dihydroartemisinin-piperaquine using pharmacological modelling. *Malaria journal*.
44. Kay K, Hodel EM, Hastings IM. 2015. Altering drug regimens to restore and enhance antimalarial drug effectiveness. *Antimicrob Agents Chemother*.
45. Hietala SF, Bhattarai A, Msellem M, Roshammar D, Ali AS, Stromberg J, et al. 2007. Population pharmacokinetics of amodiaquine and desethylamodiaquine in pediatric patients with uncomplicated falciparum malaria. *Journal of pharmacokinetics and pharmacodynamics*. doi:10.1007/s10928-007-9064-2

46. Bertrand J, Mentré F. Mathematical expressions of the pharmacokinetic and pharmacodynamic models implemented in the Monolix software. Paris Diderot University: Paris Diderot University; 2008.
47. Hodel EM, Kay K, Hayes DJ, Terlouw DJ, Hastings IM. 2014. Optimizing the programmatic deployment of the anti-malarials artemether-lumefantrine and dihydroartemisinin-piperaquine using pharmacological modelling. *Malaria journal*. doi:10.1186/1475-2875-13-138
48. Hayes DJ, van Buuren S, ter Kuile FO, Stasinopoulos DM, Rigby RA, Terlouw DJ. 2015. Developing regional weight-for-age growth references for malaria-endemic countries to optimize age-based dosing of antimalarials. *Bull World Health Organ*. doi:10.2471/blt.14.139113
49. White NJ. 2002. The assessment of antimalarial drug efficacy. *Trends in parasitology*.
50. Pradines B, Tall A, Parzy D, Spiegel A, Fusai T, Hienne R, et al. 1998. In-vitro activity of pyronaridine and amodiaquine against African isolates (Senegal) of *Plasmodium falciparum* in comparison with standard antimalarial agents. *J Antimicrob Chemother*.
51. Nawaz F, Nsobya SL, Kiggundu M, Joloba M, Rosenthal PJ. 2009. Selection of parasites with diminished drug susceptibility by amodiaquine-containing antimalarial regimens in Uganda. *J Infect Dis*. doi:10.1086/647988
52. White NJ. 1997. Assessment of the pharmacodynamic properties of antimalarial drugs in vivo. *Antimicrobial agents and chemotherapy*.
53. Stepniewska K, White NJ. 2008. Pharmacokinetic determinants of the window of selection for antimalarial drug resistance. *Antimicrobial agents and chemotherapy*. doi:10.1128/aac.00903-07
54. Michael OS, Gbotosho GO, Folarin OA, Okuboyejo T, Sowunmi A, Oduola AM, et al. 2010. Early variations in *plasmodium falciparum* dynamics in Nigerian children after treatment

- with two artemisinin-based combinations: implications on delayed parasite clearance. *Malaria journal*. doi:10.1186/1475-2875-9-335
55. Sowunmi A, Balogun T, Gbotosho GO, Happi CT, Adediji AA, Fehintola FA. 2007. Activities of amodiaquine, artesunate, and artesunate-amodiaquine against asexual- and sexual-stage parasites in falciparum malaria in children. *Antimicrobial agents and chemotherapy*. doi:10.1128/aac.00077-07
56. Fehintola FA, Adediji AA, Gbotosho GO, Happi CT, Balogun ST, Folarin OA, et al. 2008. Effects of artesunate-cotrimoxazole and amodiaquine-artesunate against asexual and sexual stages of *Plasmodium falciparum* malaria in Nigerian children. *J Infect Chemother*. doi:10.1007/s10156-008-0603-6
57. O'Neill PM, Shone AE, Stanford D, Nixon G, Asadollahy E, Park BK, et al. 2009. Synthesis, antimalarial activity, and preclinical pharmacology of a novel series of 4'-fluoro and 4'-chloro analogues of amodiaquine. Identification of a suitable "back-up" compound for N-tert-butyl isoquine. *Journal of medicinal chemistry*. doi:10.1021/jm8012757
58. Tarning J, Chotsiri P, Jullien V, Rijken MJ, Bergstrand M, Cammas M, et al. 2012. Population pharmacokinetic and pharmacodynamic modeling of amodiaquine and desethylamodiaquine in women with *Plasmodium vivax* malaria during and after pregnancy. *Antimicrobial agents and chemotherapy*. doi:10.1128/aac.01242-12
59. Brasseur P, Agnamey P, Gaye O, Vaillant M, Taylor WR, Olliaro PL. 2007. Efficacy and safety of artesunate plus amodiaquine in routine use for the treatment of uncomplicated malaria in Casamance, southern Senegal. *Malaria journal*. doi:10.1186/1475-2875-6-150
60. Gbotosho GO, Sowunmi A, Okuboyejo TM, Happi CT, Folarin OA, Michael OS, et al. 2011. Therapeutic efficacy and effects of artemether-lumefantrine and artesunate-amodiaquine

coformulated or copackaged on malaria-associated anemia in children with uncomplicated *Plasmodium falciparum* malaria in Southwest Nigeria. The American journal of tropical medicine and hygiene. doi:10.4269/ajtmh.2011.10-0582

61. Gerstl S, Dunkley S, Mukhtar A, Baker S, Maikere J. 2010. Successful introduction of artesunate combination therapy is not enough to fight malaria: results from an adherence study in Sierra Leone. Transactions of the Royal Society of Tropical Medicine and Hygiene. doi:10.1016/j.trstmh.2009.12.008

62. Beer N, Ali AS, Rotllant G, Abass AK, Omari RS, Al-mafazy AW, et al. 2009. Adherence to artesunate-amodiaquine combination therapy for uncomplicated malaria in children in Zanzibar, Tanzania. Tropical medicine & international health : TM & IH. doi:10.1111/j.1365-3156.2009.02289.x

63. Adjei GO, Kristensen K, Goka BQ, Hoegberg LC, Alifrangis M, Rodrigues OP, et al. 2008. Effect of concomitant artesunate administration and cytochrome P450C8 polymorphisms on the pharmacokinetics of amodiaquine in Ghanaian children with uncomplicated malaria. Antimicrobial agents and chemotherapy. doi:10.1128/aac.00673-07

64. Stepniewska K, Taylor W, Sirima SB, Ouedraogo EB, Ouedraogo A, Gansane A, et al. 2009. Population pharmacokinetics of artesunate and amodiaquine in African children. Malaria journal. doi:10.1186/1475-2875-8-200

65. Ali AM, Penny MA, Smith TA, Workman L, Sasi P, Adjei GO, et al. 2018. Population Pharmacokinetics of the Antimalarial Amodiaquine: a Pooled Analysis To Optimize Dosing. Antimicrobial agents and chemotherapy. doi:10.1128/aac.02193-17

66. Ajayi IO, Browne EN, Bateganya F, Yar D, Happi C, Falade CO, et al. 2008. Effectiveness of artemisinin-based combination therapy used in the context of home

management of malaria: a report from three study sites in sub-Saharan Africa. Malaria journal.
doi:10.1186/1475-2875-7-190

67. Pongtavornpinyo W, Yeung S, Hastings I, Dondorp A, Day N, White N. 2008. Spread of
anti-malarial drug resistance: Mathematical model with implications for ACT drug policies.
Malaria Journal.

68. Maire N, Smith T, Ross A, Owusu-Agyei S, Dietz K, Molineaux L. 2006. A model for
natural immunity to asexual blood stages of *Plasmodium falciparum* malaria in endemic areas.
The American journal of tropical medicine and hygiene.

69. Molineaux L, Diebner HH, Eichner M, Collins WE, Jeffery GM, Dietz K. 2001.
Plasmodium falciparum parasitaemia described by a new mathematical model. Parasitology.
doi:doi:10.1017/S0031182001007533

70. Sirima SB, Gansane A. 2007. Artesunate-amodiaquine for the treatment of
uncomplicated malaria. Expert Opin Investig Drugs. doi:10.1517/13543784.16.7.1079

71. Schramm B, Valeh P, Baudin E, Mazinda CS, Smith R, Pinoges L, et al. 2013.
Tolerability and safety of artesunate-amodiaquine and artemether-lumefantrine fixed dose
combinations for the treatment of uncomplicated *Plasmodium falciparum* malaria: two open-
label, randomized trials in Nimba County, Liberia. Malaria journal. doi:10.1186/1475-2875-12-
250

72. Adjuik M, Agnamey P, Babiker A, Borrmann S, Brasseur P, Cisse M, et al. 2002.
Amodiaquine-artesunate versus amodiaquine for uncomplicated *Plasmodium falciparum* malaria
in African children: a randomised, multicentre trial. Lancet.

73. Holmgren G, Hamrin J, Svard J, Martensson A, Gil JP, Bjorkman A. 2007. Selection of
pfmdr1 mutations after amodiaquine monotherapy and amodiaquine plus artemisinin

combination therapy in East Africa. *Infection, genetics and evolution : journal of molecular epidemiology and evolutionary genetics in infectious diseases*. doi:10.1016/j.meegid.2007.03.005

74. Mutabingwa TK, Anthony D, Heller A, Hallett R, Ahmed J, Drakeley C, et al. 2005. Amodiaquine alone, amodiaquine+sulfadoxine-pyrimethamine, amodiaquine+artesunate, and artemether-lumefantrine for outpatient treatment of malaria in Tanzanian children: a four-arm randomised effectiveness trial. *Lancet*. doi:10.1016/s0140-6736(05)66417-3

75. Bruxvoort K, Goodman C, Kachur SP, Schellenberg D. 2014. How patients take malaria treatment: a systematic review of the literature on adherence to antimalarial drugs. *PLoS One*. doi:10.1371/journal.pone.0084555

76. Cairns M, Cisse B, Sokhna C, Cames C, Simondon K, Ba EH, et al. 2010. Amodiaquine dosage and tolerability for intermittent preventive treatment to prevent malaria in children. *Antimicrobial agents and chemotherapy*. doi:10.1128/aac.01161-09

77. Barnes KI, Watkins WM, White NJ. 2008. Antimalarial dosing regimens and drug resistance. *Trends in parasitology*. doi:10.1016/j.pt.2007.11.008

78. Taylor W, Terlouw DJ, Olliaro PL, White NJ, Brasseur P, ter Kuile FO. 2006. Use of weight-for-age-data to optimize tablet strength and dosing regimens for a new fixed-dose artesunate-amodiaquine combination for treating falciparum malaria. *Bulletin of the World Health Organization*.

Figure legends

Figure 1. Structural pharmacokinetic model of artesunate (AS). AS follows a one-compartment disposition model, where it is absorbed from the gut at rate k_a and converted into dihydroartemisinin (DHA) at rate k_m . DHA is eliminated at rate k_{DHA} . Note that full conversion of AS to DHA was assumed i.e. AS was not eliminated, only converted

Abbreviations: AS: artesunate; DHA: dihydroartemisinin; V_{AS} : volume of distribution of AS; V_{DHA} ; volume of distribution of DHA. This figure was adapted from Kay and Hasting (41).

Figure 2. Structural pharmacokinetic model of amodiaquine (AQ) and desethylamodiaquine (DEAQ). The model includes two separate two-compartment models of AQ (Panel A) and its active metabolite DEAQ (Panel B). Both models share the same absorption rate (k_a) but the remaining parameters were estimated independently for AQ and DEAQ. This figure was adapted from Hietala *et al.* (45).

Abbreviations: k_a : absorption rate constant; V_{d_c} : central volume of distribution; V_{d_p} : peripheral volume of distribution; AQ: amodiaquine; DEAQ: desethylamodiaquine; $k_{AQ/DEAQ}$: elimination rate constants for AQ and DEAQ respectively; Q : distribution clearance between compartment.

Figure 3. The impact of patient adherence on treatment outcome. This was in patients prescribed regimen A (7), i.e. prescribed the therapeutic target dose of 4 mg/kg/day for artesunate and 10 mg/kg/day for amodiaquine (26) (**Table 3**). Each bar in the plots represents a decreasing proportion of fully-adherent patients in the simulated population. Non-adherent

patients were assumed to take their regimen according to **(A)** Scenario 2, i.e. missed their third treatment dose (**Table 2**), or **(B)** Scenario 3, i.e. missed both their second and third doses (**Table 2**).

Abbreviation: LoD: limit of microscopic detection; Recrude: Recrudescence

Figure 4. Distribution of parasite clearance time (PCT) and time to recrudescence (TTR) (28). PCT and TTR following treatment with artesunate-amodiaquine (7) and different scenarios (detailed in **Table 2**).

Figure 5. Treatment outcome in patients receiving age- or weight-based dosing regimens of artesunate-amodiaquine. Scenario 1 treatment outcome for regimens B to G (**Table 3**) simulated using three different region-specific weight-for-age references (Africa, Asia and Latin America). The panels represent the patients **(A)** were fully adherent (Scenario 1); **(B)** omitted one dose of AS-AQ (Scenario 2); **(C)** omitted two doses of AS-AQ (Scenario 3)

Figure 6. The percentage of patients over- or under-dosed with amodiaquine (AQ) depending on regional weight-for-age distribution. Patients were dosed according to either weight (red) or age (green) and given either a fixed dose formulation (yellow) or a co-blistered formulation (purple) of artesunate-amodiaquine. Regimens B to G were as described in **Table 3**.

790

791 **Figure 7. Amodiaquine (AQ) dosage received by simulated patients dosed according to**
 792 **weight or age.** Simulated regimens are listed in **Table 3.** **(A)** Weight-based regimens (regimens
 793 B). **(B)** Age-based regimens (regimens D). The red dotted lines indicate the therapeutic dose
 794 range of AQ (26). The blue arrows indicate the cut-off points of the age-based regimen.

795

Table 1. Antimalarial drug parameters for artesunate, amodiaquine and their active metabolites (DHA and DEAQ). The reported values are mean parameters (coefficient of variation).

Drug parameter	AS (41)	DHA (41)	AQ (45, 58)	DEAQ (45, 58)
Vd_c (L/kg)	46.6 (82)	15 (48)	8 (65)	18.4 (48)
Vd_p (L/kg)	–	–	280 (26)	68 (18)
CL (L/hr)	–	–	14 (22)	0.66 (123)
k_a (/day)	23.98 (68)	–	2.16 (30)	2.16 (30)
k_m (/day)	11.97 (65)	–	–	–
k (/day)	–	44.15 (123)	–	–
Q (L/hr)	–	–	17 (26)	1.3 (30)
IC_{50} (mg/L)	0.0023 (79)	0.009 (117)	0.0043 (163) (50)	0.02 (132) (51)
V_{max}^a (/day)	27.6	27.6	2.3 (52)	2.3 (52)
n	4	4	7 (53)	7 (53)

Abbreviations: Vd_c : central volume of distribution; Vd_p : peripheral volume of distribution; CL : clearance; k_a : absorption rate of parent drug; k_m : conversion rate to active metabolite; k : elimination rate of the drug or its metabolite; Q : distribution clearance between compartments; IC_{50} : 50% maximum inhibitory drug concentration; V_{max} : maximal parasite-killing rate constant; n : slope factor; AS: artesunate; DHA: dihydroartemisinin; AQ: amodiaquine; DEAQ: desethylamodiaquine.

^a $V_{max} = -0.5 \times \ln(1/PRR)$, where PRR is the parasite reduction ratio.

Table 2. Simulated scenarios.

Scenario	AS treatment schedule	AQ treatment schedule
1 Baseline (fully adherent)	Once daily for three days	Once daily for three days
2 Omitting the third dose	Once daily for two days	Once daily for two days
3 Omitting the second and third dose	Single dose	Single dose
4 Delaying doses for 12 hours	At 0, 36, and 60 hours	At 0, 36, and 60 hours
5 AQ monotherapy	–	Once daily for three days
6 AS monotherapy	Once daily for three days	–

Abbreviations: AQ: amodiaquine; AS: artesunate.

Table 3. Simulated dosing regimens.

	Regimen	AS dose	AQ dose
A	WHO target dose (2)	4 mg/kg	10 mg/kg
	Weight-based		
B	Study 1 – FDC (2)		
	4.5-8.9 kg	25 mg	67.5 mg
	9-17.9 kg	50 mg	135 mg
	18-35.9 kg	100 mg	270 mg
	> 36 kg	200 mg	540 mg
C	Study 1 – co-blistered (2)		
	4.5-8.9 kg	25 mg	76.5 mg
	9-17.9 kg	50 mg	153 mg
	18-35.9 kg	100 mg	306 mg
	> 36 kg	200 mg	612 mg
	Age-based		
D	Study 2 – FDC (11)		
	6-11 month	25 mg	67.5 mg
	1-5 years	50 mg	135 mg
	6-13 years	100 mg	270 mg
	≥ 14 years	200 mg	540 mg
E	Study 3 – co-blistered (59)		
	< 1 year	25 mg	76.5 mg
	1-5 years	50 mg	153 mg
	6-12 years	100 mg	306 mg
	>13years	200 mg	612 mg
F	Study 4 – FDC (60)		
	1-5 years	50 mg	135 mg
	6-13 years	100 mg	270 mg
	≥ 14 years	200 mg	540 mg
G	Study 4 – co-blistered (60)		
	1-5 years	50 mg	153 mg
	6-10 years	100 mg	306 mg
	11-15 years	150 mg	459 mg

Abbreviations: WHO: World Health Organization; AS: artesunate; AQ: amodiaquine; FDC: fixed-dose combination

Table 4. Cure rates for different combinations of dosing regimens, adherence scenarios and adherence levels. Details of scenarios and regimens can be found in **Table 2** and **Table 3**

respectively. Follow-up time was 28 days. The adherence level column indicates the proportion of simulated individuals (n=10,000) following the recommended dosing for each regimen according to scenario 1 (i.e. once daily dosing for three days). For example, when simulating scenario 2 with regimen A at an adherence level of 77%, means 77% of patients (n=7,700) followed scenario 1 with regimen A, and the remaining 23% of patients (n=2,300) missed the last dose of treatment according to scenario 2 with regimen A.

Scenario	Regimen	Adherence	Cure rate [%] per region				Safety*
		level to scenario 1 [%]	Global	Africa	Asia	Latin America	
1	A	100	96.35	-	-	-	No
	B	100	96.59	96.27	96.64	96.28	Yes
	C	100	97.19	96.99	97.28	96.93	Yes
	D	100	97.21	96.42	97.44	95.50	Yes
	E	100	97.90	97.30	98.12	96.47	Yes
	F	100	97.46	96.73	97.71	95.85	Yes
	G	100	97.16	96.24	97.44	95.31	Yes
2	A	0	80.56	-	-	-	No
		50	90.08	-	-	-	No
		60	90.83	-	-	-	No
		77	93.18	-	-	-	No
		80	93.64	-	-	-	No
		85	94.36	-	-	-	No
	B	0	80.81	79.95	81.04	79.03	No
	C	0	83.22	82.34	83.56	81.73	No
	D	0	83.53	81.04	84.56	76.81	No
	E	0	86.12	83.56	87.02	80.53	No

	F	0	84.64	79.03	85.52	78.12	No
	G	0	83.34	81.73	84.28	77.03	No
3	A	0	22.91	-	-	-	No
		60	67.17	-	-	-	No
		77	79.42	-	-	-	No
		80	81.61	-	-	-	No
		85	85.28	-	-	-	No
		90	89.00	-	-	-	No
	B	0	25.48	24.51	25.79	23.31	No
	C	0	29.34	28.40	30.17	27.56	No
	D	0	30.00	25.78	31.33	21.61	No
	E	0	34.86	30.70	36.52	26.58	No
	F	0	31.35	27.05	32.64	22.76	No
	G	0	30.95	26.88	32.42	22.86	No
4	A	0	96.30	-	-	-	No
5	A	0	35.80	-	-	-	No
6	A	0	22.88	-	-	-	No

* Indicates whether this combination of scenario and regimen was included in the safety assessment.

Table 5 Dosing accuracy and predicted exposure depending on dosing regimen. This table shows the percentage of patients from different regions receiving doses outside the therapeutic range when given one of the six regimens described in **Figure 3**, the drug dosage and predicted maximal plasma concentration of drug and its metabolites. Values of dosage and C_{max} are in medians.

	OD AS [%]	UD AS [%]	OD AQ [%]	UD AQ [%]	AS dose [mg/kg/ day]	AQ dose [mg/kg/ day]	C_{max} AS [mg/dL]	C_{max} DHA [mg/dL]	C_{max} AQ [mg/dL]	C_{max} DEAQ [mg/dL]
Regimen A										
Africa	0.0	0.0	0.0	0.0	4	10	0.5	0.8	< 0.1	0.3
Asia	0.0	0.0	0.0	0.0	4	10	0.5	0.8	< 0.1	0.3
Latin America	0.0	0.0	0.0	0.0	4	10	0.5	0.8	< 0.1	0.3
Regimen B										
Africa	0.0	0.0	0.0	1.7	3.3	8.9	0.5	0.7	< 0.1	0.3
Asia	0.0	0.0	0.0	0.0	4.2	11.3	0.5	0.8	< 0.1	0.4
Latin America	0.0	0.0	0.0	3.2	3.8	10.1	0.5	0.8	< 0.1	0.4
Regimen C										
Africa	0.0	0.0	13.8	0.4	3.9	11.8	0.5	0.8	0.1	0.4
Asia	0.0	0.0	23.2	0.0	4.2	12.8	0.5	0.8	0.1	0.4
Latin America	0.0	0.0	11.6	0.7	3.8	11.5	0.5	0.8	< 0.1	0.4
Regimen D										
Africa	0.0	0.1	9.1	6.0	4.0	10.7	0.5	0.8	< 0.1	0.4
Asia	0.0	0.0	24.0	1.4	4.8	13.0	0.6	1.0	0.1	0.4
Latin America	0.0	1.7	2.5	16.1	3.6	9.6	0.5	0.7	< 0.1	0.3

Regimen E										
Africa	0.0	0.0	23.8	1.2	4.1	12.4	0.5	0.8	0.1	0.4
Asia	0.1	0.0	50.9	0.3	4.9	15.0	0.6	1.0	0.1	0.5
Latin America	0.0	0.9	9.8	5.0	3.6	11.1	0.5	0.7	< 0.1	0.4
Regimen F										
Africa	0.1	0.0	13.6	4.1	4.1	11.1	0.5	0.8	< 0.1	0.4
Asia	0.2	0.0	29.8	0.7	5.0	13.4	0.6	1.0	0.1	0.5
Latin America	0.0	1.1	5.2	12.5	3.7	10.0	0.5	0.7	< 0.1	0.3
Regimen G										
Africa	0.1	1.2	19.4	9.1	3.6	10.9	0.5	0.7	< 0.1	0.4
Asia	0.2	0.0	32.2	0.9	4.2	12.8	0.6	0.8	0.1	0.5
Latin America	0.0	3.0	8.1	16.6	3.1	9.5	0.4	0.6	< 0.1	0.3

Abbreviations: AS: artesunate; AQ: amodiaquine; DHA: dihydroartemisinin; DEAQ: desethylamodiaquine; C_{max} : maximal concentration of drug; OD: over-dosed; UD: under-dose

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6

Figure 7