REVIEW OF ANTI-INFECTIVE AGENTS: Lou Saravolatz, Section Editor

Artemisinin Therapy for Malaria in Hemoglobinopathies: A Systematic Review

Sri Riyati Sugiarto,¹ Brioni R. Moore,^{1,2} Julie Makani,³ and Timothy M. E. Davis¹¹School of Medicine and Pharmacology, University of Western Australia, and ²School of Pharmacy, Curtin University, Perth, Western Australia, Australia; ³School of Medicine, Muhimbili University of Health and Allied Sciences, Dar es Salaam, Tanzania

Artemisinin derivatives are widely used antimalarial drugs. There is some evidence from in vitro, animal and clinical studies that hemoglobinopathies may alter their disposition and antimalarial activity. This review assesses relevant data in α -thalassemia, sickle cell disease (SCD), β -thalassemia and hemoglobin E. There is no convincing evidence that the disposition of artemisinin drugs is affected by hemoglobinopathies. Although in vitro studies indicate that *Plasmodium falciparum* cultured in thalassemic erythrocytes is relatively resistant to the artemisinin derivatives, mean 50% inhibitory concentrations (IC₅₀s) are much lower than in vivo plasma concentrations after recommended treatment doses. Since IC₅₀s are not increased in *P. falciparum* cultures using SCD erythrocytes, delayed post-treatment parasite clearance in SCD may reflect hyposplenism. As there have been no clinical studies suggesting that hemoglobinopathies significantly attenuate the efficacy of artemisinin combination therapy (ACT) in uncomplicated malaria, recommended artemisinin doses as part of ACT remain appropriate in this patient group.

Keywords. hemoglobinopathy; malaria; artemisinin derivatives; systematic review.

Hemoglobinopathies are inherited disorders characterized by alterations in the α -globin and/or β -globin components of normal adult hemoglobin (HbAA) [1]. There is either reduced production of globin chains as in α -thalassemia, β -thalassemia, and hemoglobin H, or amino acid substitutions/insertions as in hemoglobin S, hemoglobin E, and hemoglobin Constant Springs (HbCS) [1, 2] (see Table 1). Hemoglobinopathies have a geographic distribution that is influenced by malaria endemicity [2]. Gene mapping studies have shown an association between the selective pressure of *Plasmodium* infections and globin gene mutations [3]. Consistent with this finding, hemoglobinopathies offer greater protection against severe compared with uncomplicated malaria [2]. In addition, there is evidence that α -thalassemia trait, HbH, HbE, and β -thalassemia/HbE are associated with reduced parasite erythrocyte invasion [2, 4–7], while intraerythrocytic growth and maturation of malaria parasites appears to be attenuated in HbH, β -thalassemia minor, HbS, HbC, and HbE [2, 5, 7–12].

Artemisinin combination therapy (ACT), the simultaneous administration of an artemisinin derivative with a longer-acting partner drug, is the World Health Organization (WHO) recommended first-line treatment for uncomplicated malaria, with choice of ACT type based on factors such as the sensitivity of local parasites strains [13]. Artemisinin and its derivatives

dihydroartemisinin (DHA), artesunate, and artemether are sesquiterpenoids with an endoperoxide bridge that is considered to underlie their potent antimalarial effect [14]. The artemisinin component of ACT rapidly reduces parasitemia while the partner drug clears the residual parasite burden. If the response to the artemisinin drug is suboptimal, the antimalarial activity of the partner drug becomes more important in ensuring cure. However, the parasite resistance seen with conventional long half-life compounds such as chloroquine and sulfadoxine-pyrimethamine is now starting to emerge in the case of the newer partner drugs mefloquine, lumefantrine, and piperaquine [15].

There is evidence that hemoglobinopathies influence the activity of artemisinin derivatives by altering their accumulation and binding to target molecules within the parasitized erythrocyte as well as attenuating the resultant oxidative stress [16–18]. This suggests that patients with hemoglobinopathies may be at increased risk of an inadequate response to ACTs [19], especially where parasite resistance or tolerance to artemisinin drugs has emerged [20]. These are also areas in which resistance to conventional and newer partner drugs has increased the risk of ACT failure [15]. If hemoglobinopathies alter the efficacy of ACTs through an attenuated effect of the artemisinin component, further investigation into dose optimization would be justified.

The present systematic review summarizes evidence of the clinical and *in vitro* efficacy of artemisinin derivatives in the case of hemoglobinopathies.

SEARCH STRATEGY AND SELECTION CRITERIA

Relevant references were identified through searches of PubMed, MEDLINE, and Science Direct for any articles

Received 18 May 2017; editorial decision 17 August 2017; accepted 20 December 2017; published online January 23, 2018.

Correspondence: Professor T. M. E. Davis, Medical School, Faculty of Health and Medical Sciences, University of Western Australia, Fremantle Hospital, Fremantle, Western Australia 6959, Australia (tim.davis@uwa.edu.au).

Clinical Infectious Diseases® 2018;66(5):799–804

© The Author(s) 2018. Published by Oxford University Press for the Infectious Diseases Society of America. All rights reserved. For permissions, e-mail: journals.permissions@oup.com. DOI: 10.1093/cid/cix785

Table 1. Clinically Significant Hemoglobinopathies (Modified from [2])

	Genetic alteration	Epidemiology	Clinical presentation
α-chain disorders			
α^+ -thalassemia trait	Reduced globin chain production [$\alpha\alpha/\alpha^-$]	Worldwide	Asymptomatic
α^0 -thalassemia trait	Reduced globin chain production [$\alpha\alpha^-/-$]	Worldwide	Mild anemia
α -thalassemia Hemoglobin H disease (HbH)	Reduced globin chain production [$\alpha^-/-$]	Worldwide	Chronic hemolytic anemia; transfusions required to support life.
α -thalassemia Hemoglobin Constant Springs (HbCS)	Insertion of additional amino acids on one α -globin chain [$\alpha^{CS}/-$]	Southeast Asia, China	Mild anemia
α -thalassemia Hemoglobin Barts	Reduced globin chain production [$-/-$]	Worldwide	Incompatible with extra-uterine life
β-chain disorders			
β -thalassemia minor/trait	Reduced expression of one β -globin gene	Worldwide	Typically asymptomatic
β -thalassemia major	Reduced expression of both β -globin genes	Worldwide	Significant anemia leading to transfusion dependence
Hemoglobin C	Glu $\rightarrow$ Lys at position 6 of β -globin	West Africa	Mild anemia (HbCC); asymptomatic when inherited as hemoglobin C trait (HbAC)
Hemoglobin E	Glu $\rightarrow$ Lys at position 26 of β -globin	Southeast Asia	Mild anemia
Hemoglobin S	Glu $\rightarrow$ Val at position 6 of β -globin	Central/West/East Africa; South Asia; Arabian peninsula	Sickle-cell disease (HbSS, SC S β^0 Thalassemia); asymptomatic when inherited as sickle cell trait (HbAS)

including the terms “artemisinin combination therapy and hemoglobinopathy,” “antimalarial and hemoglobinopathy,” “artemisinin and thalassemia,” and “artemisinin and sickle cell.” Articles resulting from these searches and relevant references cited in those articles were reviewed. Articles published in languages other than English were eligible for inclusion if relevant. Original data have been referenced where possible, but reviews and technical reports have been cited when they offer an appropriate summary of available information.

ALPHA GLOBIN CHAIN DISORDERS

Alpha-globin chain disorders are prevalent throughout most malaria-endemic regions [21] with gene mapping studies suggesting natural selection through malaria infection [3, 22, 23]. The clinical manifestations reflect the number of deletions and/or insertions (see Table 1). Single or dual deletions (α -thalassemia trait) have an asymptomatic phenotype or manifest mild anemia, whereas patients with 3 deletions (HbH) or HbH/HbCS have more severe anemia requiring transfusions. The frequency of α -globin chain disorders is high in Southeast Asia (up to 40%) [24], a region in which multidrug parasite resistance (including to the artemisinin derivatives) is well recognized [20].

Two studies have investigated the influence of α -thalassemia on artemisinin derivative pharmacokinetics, with apparently conflicting results. The first, involving 10 healthy HbAA and 10 α -thalassemia young adult volunteers, found that exposure to artesunate and its active metabolite DHA, both mean peak concentration (C_{max}) and area under the concentration-time curve (AUCs), were at least 9 times higher in the participants with α -thalassemia after a single intravenous 2.4 mg/kg dose of artesunate as assessed by noncompartmental analysis of plasma DHA equivalent concentrations generated by bioassay [25]. The second study was conducted in 47 Melanesian children with

uncomplicated falciparum or vivax malaria, 78% of whom had at least one α -thalassemia gene deletion, who were treated with 2 doses of rectal artesunate (10 to 15 mg/kg) 12 hours apart [26]. In a population pharmacokinetic model of plasma artesunate and DHA concentrations assayed by liquid chromatography-mass spectrometry (LC-MS), α -thalassemia status was not an independent determinant of primary or secondary pharmacokinetic parameters when added to the model as a covariate [26].

The apparent discrepancies between the findings of these 2 studies could reflect differences in the age of the participants, the route of administration, the presence/absence of malaria infection, the severity of the effects of the genetic defect (mean hematocrit 27% [25] versus 34% [26]), the drug assay methodologies, and/or the pharmacokinetic analyses used. It is possible, for example, that, in febrile Melanesian children with higher hematocrits than those of the otherwise healthy adults with α -thalassemia in the volunteer study (consistent with 46% [26] versus 100% [25] of participants with 2 or more α -globin gene deletions/mutations) who were given two doses of variably absorbed artesunate suppositories versus a single intravenous injection, the pharmacokinetic effect of α -thalassemia was less marked and more difficult to detect. However, a more likely explanation is that the plasma concentration profile in the healthy HbAA adults [25] was unexpectedly low.

Artesunate is quantitatively metabolized to DHA within minutes after intravenous injection [27]. Most pharmacokinetic studies (including the volunteer study [25]) have employed sampling schedules that have started when artesunate concentrations have declined substantially and the DHA C_{max} has been reached (≥ 10 minutes post-injection [27]), and so the disposition of the more potent DHA has been the primary focus. In a pooled analysis of published data relating to the pharmacokinetics of intravenous artesunate in 266 adults and children with

severe malaria, the median DHA $C_{\max}$ after the recommended 2.4 mg/kg dose as measured by high performance liquid chromatography or LC-MS was 9 $\mu\text{mol/L}$ (3 $\mu\text{g/L}$) [28]. The participants with α -thalassemia in the volunteer study had a lower mean DHA $C_{\max}$ of 5 $\mu\text{mol/L}$, consistent with lower first pass clearance and thus greater DHA exposure in malaria [29] but still comfortably above the 2.5th lower confidence interval for the pooled analysis (3.5 $\mu\text{mol/L}$; see Figure 1) [28]. The healthy HbAA subjects had a mean DHA $C_{\max}$ of only 0.3 $\mu\text{mol/L}$ [25], which appears at odds with all other available DHA pharmacokinetic data after intravenous artesunate [28, 30]. It is possible that accidental under-dosing or an unidentified technical issue with the bioassay, which should provide comparable plasma concentrations to chemical assays [31], may have been responsible for the apparently low DHA $C_{\max}$ in the HbAA group and which would have influenced some of the other pharmacokinetic parameters.

A further indication of the inconsistency of the DHA exposure data in the volunteer study is the conclusion that the smaller steady-state volume of distribution (V_{ss}) in α -thalassemia reflected increased erythrocyte DHA uptake [25] as shown in vitro [18, 19]. This phenomenon would, however, likely result in lower rather than higher plasma concentrations in α -thalassemia as well as a higher V_{ss} , a parameter that would be sensitive to errors in plasma DHA concentrations. The suggestion that there is a slow release of artesunate and DHA into plasma from the enriched α -thalassemic erythrocyte compartment is also inconsistent with the finding that the terminal elimination half-life for DHA, a pharmacokinetic parameter that would not have

been influenced by a systematic error in assayed plasma concentrations, was similar in the 2 groups [25]. We conclude, based on these considerations, that there is no convincing evidence that α -thalassemia alters the disposition of artemisinin drugs.

The question of whether, irrespective of pharmacokinetic considerations, α -thalassemia influences the response to artemisinin derivatives has been investigated in *ex vivo* experiments. In studies of cultured *P. falciparum*, higher media concentrations of artemisinin drugs were required to kill parasites in HbH and HbH/HbCS versus HbAA erythrocytes, with mean 50% inhibitory concentrations (IC_{50} s) of 4.5, 8.5, and 0.8 nmol/L, respectively, for artesunate [22], and 9.6, 13.7, and 1.2 nmol/L, respectively, for DHA [18]. The same pattern was found in an *ex vivo* assessment of the less potent parent artemisinin [32], with IC_{50} s of 42.3, 62.5, and 3.6 nmol/L for HbH, HbH/CS, and HbAA, respectively. These differences reflect several likely mechanisms including competition between the greater uninfected erythrocyte drug uptake and that of parasitized cells containing variant hemoglobins [18, 19] and drug inactivation by membrane and especially cytosolic components such as free heme and catalase [18, 32, 33].

Although consistently demonstrated and supported by plausible underlying mechanisms, the clinical relevance of *ex vivo* artemisinin resistance of parasitized α -thalassemic red cells is uncertain. The DHA IC_{50} values in α -thalassemic erythrocytes are an order of magnitude lower than *in vivo* plasma concentrations after recommended doses (in the low nmol/L range [18, 22] compared with $>1 \mu\text{mol/L}$ for at least several hours post-dose regardless of route of administration [26, 28]). Although a clear relationship between *ex vivo* and *in vivo* drug sensitivity has not been defined, the *in vitro* ring-stage survival assay (RSA) utilizes what is considered a pharmacologically relevant DHA concentration of 700 nmol/L to assess the survival of young parasites [34], a threshold that is also well above DHA IC_{50} s in α -thalassemic erythrocytes [18, 22]. Consistent with the proposition that mild resistance of α -thalassemic parasitized cells to DHA has little clinical relevance, there was no evidence in the Melanesian pediatric study that α -thalassemia status impaired parasite or fever clearance [26].

In summary, there are no conclusive pharmacokinetic or pharmacodynamic reasons why conventional doses of artemisinin drugs cannot be given with confidence as part of recommended ACT regimens, or as initial parenteral monotherapy in severe malaria, to patients with α -thalassemia. The emergence of artemisinin-resistant *P. falciparum* with the K13-propeller mutation in Cambodia and its subsequent spread [35] may, however, have implications in α -thalassemia since the associated DHA IC_{50} [36] and ability of young parasite forms to survive pharmacological DHA exposure as assessed in the RSA [37] may be augmented in α -thalassemic patients. Further *ex vivo* and *in vivo* studies are needed to assess this increasingly likely clinical scenario.

Figure 1. Maximum plasma dihydroartemisinin concentrations after 2.4 mg/kg artesunate given intravenously to 266 patients with severe malaria (median and 2.5th to 97.5th percentiles) [27], and to 20 healthy volunteers with α -thalassemia or normal hemoglobin (HbAA) (mean and absolute range) [24]. The shaded area represents the 50% inhibitory concentration for DHA at 2 standard deviations above the mean for *Plasmodium falciparum* cultured in α -thalassemic erythrocytes.

BETA GLOBIN CHAIN DISORDERS

Sickle Cell Disease

Sickle cell disease (SCD) refers to a group of disorders caused by β -globin gene mutations that produce sickle-shaped erythrocytes. They include the homozygous pattern (HbSS; sickle cell anemia) and the combination of a single mutation with another abnormal β globin gene allele (SC, S- β^0 thalassemia). Sickled cells are more easily eliminated by the reticuloendothelial system and more readily undergo hemolysis, leading to anemia. Patients with SCD can either spontaneously or in response to stress such as infections (including malaria) undergo rapid decompensation and develop an acute clinical event or "crisis." SCD is the most common monogenic disease globally with its highest prevalence in malaria-endemic regions of sub-Saharan Africa [38], reflecting the selection pressure associated with malaria infection [39].

Individuals heterozygous for β -globin gene mutations have sickle cell trait (SCT or HbAS) with a lower number of circulating sickled erythrocytes. Although SCT erythrocytes have increased fragility, patients are not typically anemic and develop symptoms only if they are severely hypoxic or dehydrated. There is strong evidence for protection against malaria in SCT [40, 41], but the evidence in SCD is less consistent [2]. Malarial parasitemia has an adverse prognosis in SCD including substantially increased mortality [42]. It is therefore recommended that individuals with SCD in malaria-endemic countries should receive antimalarial chemoprophylaxis [43].

There have been no pharmacokinetic studies of ACTs in patients with SCD or SCT, but 2 African studies have included children with SCT [44] or SCD [45] in a comparison of the efficacy of artemether-lumefantrine (AL) and artesunate-amodiaquine (AS-AQ) for uncomplicated falciparum malaria. In the first, 41 of 266 Congolese children aged <10 years (15.6%) had SCT and the remainder HbAA [44]. The 28-day adequate clinical and parasitological response (ACPR) was >95% regardless of allocated therapy and hemoglobin subgroup. Although parasite and fever clearance times were not recorded, there were no cases of early treatment failure [44], suggesting that SCT was not associated with an initial increase in parasitemia or prolonged parasite clearance beyond the second day of treatment. The authors concluded that SCT did not alter the efficacy of either ACT, consistent with an ex vivo study showing similar artemisinin IC_{50} s against *P. falciparum* cultured in SCT and HbAA erythrocytes [46].

In the second efficacy study involving 60 Ghanaian children with SCD (HbSS) and 59 with HbAA [45], the 42-day ACPR was >96% regardless of allocated therapy and hemoglobinopathy subgroup. The times to 50% and 90% parasite clearance based on group geometric mean densities were 2 and 5 days, respectively, in SCD and 0.6 and 1.6 days, respectively, in the children with HbAA. The authors further analyzed the data in an attempt to allow for the much lower baseline parasitemia in

the SCD group with the result that an effect of SCD on clearance was no longer seen, but they concluded that the lower clearance rate in SCD despite a lower initial parasitemia suggested a true between-group difference.

The overall interpretation of these two clinical studies includes several important points. First, WHO-recommended ACTs are efficacious in SCT and SCD. Although the lower baseline parasitemia in the SCD patients is consistent with the protective effect of HbSS due to impaired intra-erythrocytic parasite development [47, 48], any malaria parasitemia in SCD is associated with increased morbidity and mortality [42]. Therefore, malaria complicating SCD should be treated promptly. Second, although the slower parasite clearance in SCD may be difficult to interpret when the baseline parasite density is low [45], it is nevertheless consistent with the hyposplenism that develops at a young age (<12 months) in SCD [49]. Delayed parasite clearance is well described in patients who have undergone splenectomy, including in those treated with repeated courses of ACTs [50]. Third, whether the parasites that circulate for many days after treatment in SCD and other hyposplenic states are viable is uncertain [50], but the lack of an excess of late clinical and parasitologic failure in SCT/SCD children in the Congolese and Ghanaian studies is reassuring.

Beta-thalassemia

The β -thalassemia disorders arise from reduced expression of one or both β -globin genes (see Table 1). A single β -globin gene mutation/deletion results in β -thalassemia trait or β -thalassemia minor, a condition which may afford some protection against falciparum malaria [51]. Dual β -globin gene abnormalities (β -thalassemia major) are associated severe transfusion-dependent anemia. Studies of ACTs in β -thalassemia have been limited to ex vivo experiments investigating the role of erythrocyte age, oxidant stress and membrane heme on the activity of the artemisinin derivatives [52, 53]. Cultured *P. falciparum* in β -thalassemia trait erythrocytes showed higher IC_{50} s to both artemether [52] and artemisinin [54] compared with HbAA erythrocyte cultures, especially in the case of chloroquine-sensitive parasites and in older red cells. Because the increase in IC_{50} was of similar magnitude to that associated with α -thalassemia trait erythrocytes in the same culture system with both well below in vivo plasma concentrations after recommended treatment doses, the clinical relevance of these findings is uncertain.

Hemoglobin E

Hemoglobin E (HbE) is a common hemoglobinopathy throughout South-east Asia, with a highest prevalence of 25% in areas of Thailand [55]. Although not considered a thalassemia, erythrocytes from patients with HbE disease resemble β -thalassemic red cells. A pooled analysis of studies conducted in Myanmar [56] and Thailand [55] did not show evidence of protection

against malaria, but the findings of the 2 studies were heterogeneous [2].

There have been no pharmacokinetic studies of ACTs in patients with HbE, but the efficacy of the artemisinin derivatives has been assessed through analysis of data from Thai clinical trial participants [55] and the effectiveness of artesunate-mefloquine has been evaluated in a more recent Cambodian study [57]. In the Thai study, parasite clearance was faster in patients with HbE trait treated with DHA, artesunate or artemether than in the same number of control patients who were negative for HbE, β thalassemia, HbH, and HbCS (medians 38 vs 50 hours, respectively) while there was no such difference in the case of other antimalarial regimens (mefloquine or quinine with or without tetracycline or doxycycline) [55]. In the Cambodian study, conducted in an area of emerging artemisinin-resistant *P. falciparum*, there was a nonsignificant mean 0.6 hour prolongation of the parasite clearance half-life in patients with HbE compared to non-HbE participants [57].

Several explanations for the HbE-associated accelerated parasite clearance were suggested by the authors of the Thai study, including the pro-oxidant effect of degradation of the increased amounts of α -globin chains and possible alterations in artemisinin drug disposition in HbE trait [55]. It is also possible that variant hemoglobins such as HbE may modulate the response of resistant *P. falciparum* strains to artemisinin drugs [57], as in the case of α -thalassemia. However, in addition to the apparently contrasting influences of HbE on parasite clearance in these 2 studies, the magnitude of these effects was of limited clinical significance.

CONCLUSION

The effects of the most common hemoglobinopathies on the response to treatment with artemisinin drugs, whether given alone or as part of ACT for uncomplicated malaria, do not appear to be clinically significant based on available in vivo and ex vivo data. Patients with undiagnosed hemoglobinopathies should respond appropriately to ACTs without the risk of increased adverse effects. Patients with known hemoglobinopathies may require increased monitoring for anemia, hemolysis and, in the case of SCD, delayed parasite clearance. The latter phenomenon is consistent with SCD-associated hyposplenism. There is no evidence relating to the efficacy of ACTs when used as intermittent presumptive therapy of malaria in hemoglobinopathies. The emergence of artemisinin-resistant *P. falciparum* may unmask important interactions between hemoglobinopathies and treatment failure, and should be the subject of ongoing research.

Notes

Financial support. This work was supported by an Australia Award Scholarship from the Australian Government's Department of Foreign Affairs and Trade Australian Government (to S. R. S.). and a National

Health and Medical Research Council of Australia Practitioner Fellowship (1058260 to T. M. E. D.).

Potential conflicts of interest. All authors: No reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Trent RJA. Diagnosis of the haemoglobinopathies. Clin Biochem Rev 2006; 27: 27–38.
2. Taylor SM, Parobek CM, Fairhurst RM. Haemoglobinopathies and the clinical epidemiology of malaria: a systematic review and meta-analysis. Lancet Infect Dis 2012; 12:457–68.
3. Flint J, Hill AV, Bowden DK, et al. High frequencies of alpha-thalassaemia are the result of natural selection by malaria. Nature 1986; 321:744–50.
4. Bunyaratvej A, Butthep P, Sae-Ung N, Fucharoen S, Yuthavong Y. Reduced deformability of thalassemic erythrocytes and erythrocytes with abnormal hemoglobins and relation with susceptibility to *Plasmodium falciparum* invasion. Blood 1992; 79:2460–3.
5. Ifediba TC, Stern A, Ibrahim A, Rieder RF. *Plasmodium falciparum* in vitro: diminished growth in hemoglobin H disease erythrocytes. Blood 1985; 65:452–5.
6. Chotivanich K, Udomsangpetch R, Pattanapanyasat K, et al. Hemoglobin E: a balanced polymorphism protective against high parasitemias and thus severe *P. falciparum* malaria. Blood 2002; 100:1172–6.
7. Brockelman CR, Wongsattayanont B, Tan-ariya P, Fucharoen S. Thalassemic erythrocytes inhibit in vitro growth of *Plasmodium falciparum*. J Clin Microbiol 1987; 25:56–60.
8. Pasvol G. The interaction between sickle haemoglobin and the malarial parasite *Plasmodium falciparum*. Trans R Soc Trop Med Hyg 1980; 74:701–5.
9. Friedman MJ, Roth EF, Nagel RL, Trager W. The role of hemoglobins C, S, and Nalt in the inhibition of malaria parasite development in vitro. Am J Trop Med Hyg 1979; 28:777–80.
10. Fairhurst RM, Fujioka H, Hayton K, Collins KF, Welles TE. Aberrant development of *Plasmodium falciparum* in hemoglobin CC red cells: implications for the malaria protective effect of the homozygous state. Blood 2003; 101:3309–15.
11. Nagel RL, Raventos-Suarez C, Fabry ME, Tanowitz H, Sicard D, Labie D. Impairment of the growth of *Plasmodium falciparum* in HbEE erythrocytes. J Clin Invest 1981; 68:303–5.
12. Wilson RJ, Pasvol G, Weatherall DJ. Invasion and growth of *Plasmodium falciparum* in different types of human erythrocyte. Bull World Health Organ 1977; 55:179–86.
13. World Health Organization. Guidelines for the treatment of Malaria. Geneva: World Health Organization, 2015.
14. Cui L, Su XZ. Discovery, mechanisms of action and combination therapy of artemisinin. Expert Rev Anti Infect Ther 2009; 7:999–1013.
15. White NJ. Can new treatment developments combat resistance in malaria? Expert Opin Pharmacother 2016; 17:1303–7.
16. Friedman MJ. Oxidant damage mediates variant red cell resistance to malaria. Nature 1979; 280: 245–7.
17. Prasartkaew S, Bunyaratvej A, Fucharoen S, Wasi P. Comparison of erythrocyte antioxidative enzyme activities between two types of haemoglobin H disease. J Clin Pathol 1986; 39:1299–303.
18. Vattanaviboon P, Wilairat P, Yuthavong Y. Binding of dihydroartemisinin to hemoglobin H: role in drug accumulation and host-induced antimalarial ineffectiveness of alpha-thalassemic erythrocytes. Mol Pharmacol 1998; 53:492–6.
19. Kamchonwongpaisan S, Chandra-ngam G, Avery MA, Yuthavong Y. Resistance to artemisinin of malaria parasites (*Plasmodium falciparum*) infecting alpha-thalassemic erythrocytes in vitro: competition in drug accumulation with uninfected erythrocytes. J Clin Invest 1994; 93:467–73.
20. Woodrow CJ, White NJ. The clinical impact of artemisinin resistance in Southeast Asia and the potential for future spread. FEMS Microbiol Rev 2017; 41:34–48.
21. World Health Organization. World Malaria Report. Geneva: World Health Organization, 2015.
22. Yuthavong Y, Butthep P, Bunyaratvej A, Fucharoen S. Decreased sensitivity of artesunate and chloroquine of *Plasmodium falciparum* infecting hemoglobin H and/or hemoglobin constant spring erythrocytes. J Clin Invest 1989; 83:502–5.
23. Hedrick PW. Resistance to malaria in humans: the impact of strong, recent selection. Malar J 2012; 11:349.
24. Fucharoen S, Winichagoon P. Haemoglobinopathies in southeast Asia. Indian J Med Res 2011; 134:498–506.
25. Ittarat W, Looareesuwan S, Pootrakul P, Sumpunsirikul P, Vattanavibool P, Meshnick SR. Effects of alpha-thalassemia on pharmacokinetics of the antimalarial agent artesunate. Antimicrob Agents Chemother 1998; 42:2332–5.

26. Karunajeewa HA, Ilett KF, Dufall K, et al. Disposition of artesunate and dihydroartemisinin after administration of artesunate suppositories in children from Papua New Guinea with uncomplicated malaria. *Antimicrob Agents Chemother* **2004**; 48:2966–72.
27. Davis TM, Phuon HL, Ilett KF, et al. Pharmacokinetics and pharmacodynamics of intravenous artesunate in severe falciparum malaria. *Antimicrob Agents Chemother* **2001**; 45:181–6.
28. Zaloumis SG, Tarning J, Krishna S, et al. Population pharmacokinetics of intravenous artesunate: a pooled analysis of individual data from patients with severe malaria. *CPT Pharmacometrics Syst Pharmacol* **2014**; 3:e145.
29. Binh TQ, Ilett KF, Batty KT, et al. Oral bioavailability of dihydroartemisinin in Vietnamese volunteers and in patients with falciparum malaria. *Br J Clin Pharmacol* **2001**; 51:541–6.
30. Davis TM, Breheny FX, Kendall PA, et al. Severe falciparum malaria with hyperparasitaemia treated with intravenous artesunate. *Med J Aust* **1997**; 166:416–8.
31. Kotecka BM, Rieckmann KH, Davis TM, Batty KT, Ilett KF. Comparison of bioassay and high performance liquid chromatographic assay of artesunate and dihydroartemisinin in plasma. *Acta Trop* **2003**; 87:371–5.
32. Charoentearaboon J, Kamchonwongpaisan S, Wilairat P, Vattanaviboon P, Yuthavong Y. Inactivation of artemisinin by thalassemic erythrocytes. *Biochem Pharmacol* **2000**; 59:1337–44.
33. Pongmee N, Chuchue T, Wilairat P, Yuthavong Y, Kamchonwongpaisan S. Artemisinin effectiveness in erythrocytes is reduced by heme and heme-containing proteins. *Biochem Pharmacol* **2007**; 74:153–60.
34. Witkowski B, Amaratunga C, Khim N, et al. Novel phenotypic assays for the detection of artemisinin-resistant *Plasmodium falciparum* malaria in Cambodia: in-vitro and ex-vivo drug-response studies. *Lancet Infect Dis* **2013**; 13:1043–9.
35. Ashley EA, Dhorda M, Fairhurst RM, et al.; Tracking Resistance to Artemisinin Collaboration (TRAC). Spread of artemisinin resistance in *Plasmodium falciparum* malaria. *N Engl J Med* **2014**; 371:411–23.
36. Noedl H, Socheat D, Satimai W. Artemisinin-resistant malaria in Asia. *N Engl J Med* **2009**; 361:540–1.
37. Arie E, Witkowski B, Amaratunga C, et al. A molecular marker of artemisinin-resistant *Plasmodium falciparum* malaria. *Nature* **2014**; 505:50–5.
38. Piel FB, Patil AP, Howes RE, et al. Global epidemiology of sickle haemoglobin in neonates: a contemporary geostatistical model-based map and population estimates. *Lancet* **2013**; 381:142–51.
39. Malaria Genomic Epidemiology Network. Reappraisal of known malaria resistance loci in a large multicenter study. *Nat Genet* **2014**; 46: 1197–204.
40. Aidoo M, Terlouw DJ, Kolczak MS, et al. Protective effects of the sickle cell gene against malaria morbidity and mortality. *Lancet* **2002**; 359:1311–2.
41. ALLISON AC. Protection afforded by sickle-cell trait against subtertian malarial infection. *Br Med J* **1954**; 1:290–4.
42. Makani J, Komba AN, Cox SE, et al. Malaria in patients with sickle cell anemia: burden, risk factors, and outcome at the outpatient clinic and during hospitalization. *Blood* **2010**; 115:215–20.
43. Oniyangi O, Omari AA. Malaria chemoprophylaxis in sickle cell disease. *Cochrane Database Syst Rev* **2006**; 4: CD003489.
44. Ndounga M, Pembe Issamou Mayengue, Casimiro PN, et al. Artesunate-amodiaquine versus artemether-lumefantrine for the treatment of acute uncomplicated malaria in Congolese children under 10 years old living in a suburban area: a randomized study. *Malar J* **2015**; 14:423.
45. Adjei GO, Goka BQ, Enweronu-Laryea CC, et al. A randomized trial of artesunate-amodiaquine versus artemether-lumefantrine in Ghanaian paediatric sickle cell and non-sickle cell disease patients with acute uncomplicated malaria. *Malar J* **2014**; 13:369.
46. Walcourt A, Kurantsin-Mills J, Adenuga BB, Loyevsky M, Kassim OO, Gordeuk VR. *Plasmodium falciparum*: activity of artemisinin against *Plasmodium falciparum* cultured in sickle trait hemoglobin AS and normal hemoglobin AA red blood cells. *Exp Parasitol* **2008**; 120:381–4.
47. Tiffert T, Lew VL, Ginsburg H, Krugliak M, Croisille L, Mohandas N. The hydration state of human red blood cells and their susceptibility to invasion by *Plasmodium falciparum*. *Blood* **2005**; 105:4853–60.
48. Williams TN, Obaro SK. Sickle cell disease and malaria morbidity: a tale with two tails. *Trends Parasitol* **2011**; 27:315–20.
49. Brousse V, Buffet P, Rees D. The spleen and sickle cell disease: the sick(led) spleen. *Br J Haematol* **2014**; 166:165–76.
50. Le TA, Davis TM, Tran QB, Nguyen VP, Trinh KA. Delayed parasite clearance in a splenectomized patient with falciparum malaria who was treated with artemisinin derivatives. *Clin Infect Dis* **1997**; 25:923–5.
51. Willcox M, Björkman A, Brohult J, Pehrson PO, Rombo L, Bengtsson E. A case-control study in northern Liberia of *Plasmodium falciparum* malaria in haemoglobin S and beta-thalassaemia traits. *Ann Trop Med Parasitol* **1983**; 77:239–46.
52. Senok AC, Li K, Nelson EA, Chung KW. *In vitro* sensitivity of artemeter in *Plasmodium falciparum*-infected beta-thalassaemic trait erythrocytes. *Parasitology* **1999**; 118 (Pt 2):145–9.
53. Vattanaviboon P, Siritanaratkul N, Ketpirune J, Wilairat P, Yuthavong Y. Membrane heme as a host factor in reducing effectiveness of dihydroartemisinin. *Biochem Pharmacol* **2002**; 64:91–8.
54. Senok AC, Nelson EA, Li K, Oppenheimer SJ. Thalassaemia trait, red blood cell age and oxidant stress: effects on *Plasmodium falciparum* growth and sensitivity to artemisinin. *Trans R Soc Trop Med Hyg* **1997**; 91:585–9.
55. Hutagalung R, Wilairatana P, Looareesuwan S, Brittenham GM, Gordeuk VR. Influence of hemoglobin E trait on the antimalarial effect of artemisinin derivatives. *J Infect Dis* **2000**; 181:1513–6.
56. Oo M, Tin-Shwe, Marlar-Than, O'Sullivan WJ. Genetic red cell disorders and severity of falciparum malaria in Myanmar. *Bull World Health Organ* **1995**; 73:659–65.
57. Amaratunga C, Sreng S, Suon S, et al. Artemisinin-resistant *Plasmodium falciparum* in Pursat province, western Cambodia: a parasite clearance rate study. *Lancet Infect Dis* **2012**; 12:851–8.