

Antimalarial Drug Resistance: Literature Review and Activities and Findings of the ICEMR Network

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Abstract. Antimalarial drugs are key tools for the control and elimination of malaria. Recent decreases in the global malaria burden are likely due, in part, to the deployment of artemisinin-based combination therapies. Therefore, the emergence and potential spread of artemisinin-resistant parasites in southeast Asia and changes in sensitivities to artemisinin partner drugs have raised concerns. In recognition of this urgent threat, the International Centers of Excellence for Malaria Research (ICEMRs) are closely monitoring antimalarial drug efficacy and studying the mechanisms underlying drug resistance. At multiple sentinel sites of the global ICEMR network, research activities include clinical studies to track the efficacies of antimalarial drugs, ex vivo/in vitro assays to measure drug susceptibilities of parasite isolates, and characterization of resistance-mediating parasite polymorphisms. Taken together, these efforts offer an increasingly comprehensive assessment of the efficacies of antimalarial therapies, and enable us to predict the emergence of drug resistance and to guide local antimalarial drug policies. Here we briefly review worldwide antimalarial drug resistance concerns, summarize research activities of the ICEMRs related to drug resistance, and assess the global impacts of the ICEMR programs.

INTRODUCTION

Despite important gains in some areas, malaria remains a major problem in most of the tropical world, and it continues to cause hundreds of millions of illnesses and hundreds of thousands of deaths each year.¹ Most serious illnesses and deaths from malaria and also most drug-resistant infections are due to infection with *Plasmodium falciparum*, the most virulent human malaria parasite. In addition, there is increasing appreciation of the importance of *Plasmodium vivax*, the other common human malaria parasite, as a cause of serious illnesses, and its resistance to antimalarial drugs is now well described.²

The control and eventual eradication of malaria depend on a small set of tools. For control of anopheline mosquito vectors the values of insecticide-impregnated bednets and indoor residual spraying of insecticides have been clearly demonstrated,³ but their efficacy will be limited without coincident efforts directed against malaria parasites. An effective vaccine against malaria would be extremely valuable. Unfortunately, although the RTS,S vaccine, which has offered modest protection against malaria in African children,⁴ may be available in a few years, no highly effective vaccine is on the horizon. Thus, appropriate use of antimalarial drugs remains a cornerstone of malaria control. Drugs have two key roles for malaria control. First, prompt and effective treatment of malaria prevents progression to severe disease and limits the development of gametocytes, thus blocking transmission to mosquitoes.⁵ Second, drugs can be used to prevent malaria in endemic populations, including various strategies of chemoprophylaxis, intermittent preventive therapy, and mass drug administration.⁶

The ICEMR network includes 10 groups focused on malaria surveillance and related activities in 10 different malaria-

endemic regions. This article discusses current knowledge of antimalarial drug resistance, including activities of ICEMR groups to assess and characterize resistance in different regions.

ANTIMALARIAL DRUGS

Antimalarial drugs act principally to eliminate the erythrocytic stages of malaria parasites that are responsible for human illness. Drug regimens for treatment of the two most prevalent malaria parasites, *P. falciparum* and *P. vivax*, are different. With frequent resistance to older drugs, artemisinin-based combination therapy (ACT) is now recommended for the treatment of uncomplicated falciparum malaria in nearly all areas.⁷ Chloroquine plus primaquine remains the first-line regimen for radical cure of vivax malaria in most regions. ACT consists of a potent artemisinin component, which rapidly clears most parasites, plus a longer acting partner drug, which eliminates remaining parasites and limits selection of artemisinin resistance.⁷ The ACTs recommended by the World Health Organization (WHO) are artemether/lumefantrine, artesunate/amodiaquine, artesunate/mefloquine, dihydroartemisinin/piper-aquine, artesunate/pyronaridine, and artesunate/sulfadoxine-pyrimethamine. ACTs are also effective against erythrocytic stages of non-falciparum malaria parasites. Multiple drugs are used to prevent malaria. Recommendations for travelers from nonendemic to endemic areas generally advocate use of atovaquone/proguanil (Malarone), mefloquine, or doxycycline in chemoprophylactic regimens.⁸ In Africa, intermittent preventive therapy is advocated in some high-risk populations, including sulfadoxine/pyrimethamine during pregnancy and amodiaquine/sulfadoxine-pyrimethamine as seasonal malaria chemoprophylaxis in the Sahel subregion, where there is little resistance to these drugs.⁶

Available antimalarial drugs can be divided into multiple classes (Table 1). The 4-aminoquinoline, chloroquine, was the gold standard for the treatment of uncomplicated malaria for many years, but it is no longer appropriate for the treatment of falciparum malaria in nearly all areas because of drug resistance. In Indonesia, increased resistance to chloroquine in

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TABLE 1
Currently used antimalarial drugs

| Class | Drug | Use |
|-------------------------------------|---|---|
| 4-Aminoquinoline | Chloroquine Amodiaquine Piperaquine | Treatment of non-falciparum malaria Partner drug for ACT ACT partner drug with dihydroartemisinin as ACT |
| 8-Aminoquinoline | Primaquine | Radical cure and terminal prophylaxis of <i>Plasmodium vivax</i> and <i>Plasmodium ovale</i> ; gametocytocidal drug for <i>Plasmodium falciparum</i> Radical cure of <i>P. vivax</i> and <i>P. ovale</i> |
| Arylamino alcohol | Quinine Mefloquine Lumefantrine | Treatment of <i>P. falciparum</i> and severe malaria Prophylaxis and partner drug for ACT for treatment of falciparum Combination with artemether as ACT |
| Sesquiterpene lactone endoperoxides | Artemether Artesunate | ACT: combination with lumefantrine ACT; treatment of severe malaria |
| Mannich base | Dihydroartemisinin | ACT: combination with piperaquine |
| Antifolate | Pyronaridine Pyrimethamine/sulfadoxine | Combination with artesunate as ACT Treatment of some chloroquine-resistant parasites; Combination with artesunate as ACT |
| Naphthoquinone/antifolate | Atovaquone/proguanil | Combination for prophylaxis and treatment of <i>P. falciparum</i> (Malarone) |
| Antibiotic | Doxycycline Clindamycin | Chemoprophylaxis; treatment of <i>P. falciparum</i> |

ACT = artemisinin-based combination therapy.

P. vivax prompted a policy change to ACTs for vivax malaria.⁹ Amodiaquine appears to be subject to the same resistance mechanisms as chloroquine, but due to improved potency it provides adequate efficacy against many chloroquine-resistant parasites, and it is a component of the widely used ACT artesunate/amodiaquine. A third 4-aminoquinoline, piperaquine, was widely used to treat and prevent malaria in China a few decades ago, but it then fell into disfavor because of increasing drug resistance.¹⁰ More recently, piperaquine has become a component of another ACT, dihydroartemisinin/piperaquine. The 8-aminoquinoline, primaquine, has some activity against erythrocytic parasites, but it is used principally to eliminate parasite liver stages, including the exoerythrocytic forms that precede erythrocytic infection in all species and the hypnozoites that cause latent infections with *P. vivax* and *Plasmodium ovale*. Primaquine also acts against gametocytes, thereby lowering transmission of parasites to mosquito vectors. Quinine is an arylamino alcohol that is the oldest antimalarial drug, used as cinchona bark since the 1600s and in its pure form since 1820.¹¹ Quinine is quite hard to tolerate, and its use is best limited to the treatment of severe malaria. Important malaria-related drugs are mefloquine and lumefantrine, components of the ACTs artesunate/mefloquine and artemether/lumefantrine.

Antifolates target parasite dihydrofolate reductase (DHFR) and dihydropteroate synthase (DHPS). Sulfadoxine/pyrimethamine has the distinct advantage of single-dose therapy, but its treatment efficacy is seriously limited by drug resistance. The naphthoquinone atovaquone acts against the mitochondrial cytochrome bc₁ complex. Combined with the DHFR inhibitor proguanil as Malarone, it offers effective albeit expensive therapy and chemoprophylaxis for falciparum malaria, although it is noteworthy that the synergy of this combination appears to be independent of inhibition of folate synthesis. Several antibiotics that are prokaryotic protein synthesis inhibitors have antimalarial activity because of action against the protein synthesis machinery of the apicoplast organelle.¹² Doxycycline is used for chemoprophylaxis against malaria, and doxycycline or clindamycin are combined with quinine to treat falciparum malaria.

The most important new class of antimalarials is the artemisinins, which were developed from a natural product remedy in China.¹³ Artemisinin is a potent antimalarial, but the

derivatives artesunate, artemether, and dihydroartemisinin are most widely used as components of ACT regimens. Indeed, the use of artemisinins outside combination regimens is strongly discouraged by the WHO because of fear of selecting for resistance to this important class of drugs. Artemisinins are highly effective against acute malaria, but short acting, so combination with longer-acting drugs in ACTs allows short (3-day) courses of treatment that protect against the selection of resistance to the artemisinin component.⁷ Because of its rapid action, intravenous artesunate is also the new gold standard for the treatment of severe falciparum malaria, with documented survival advantages compared with intravenous quinine.^{14,15}

ANTIMALARIAL DRUG RESISTANCE

The efficacies of many antimalarial drugs are limited by drug resistance, and recent evidence suggests that parasites are becoming resistant to the newest agents. However, the extent of resistance varies, such that in many cases drugs with resistance concerns are nonetheless offering good effectiveness for the treatment and control of malaria. Resistance has been described for nearly all available drugs, as is discussed below. For many drugs the extent of resistance is uncertain and mechanisms of resistance are unknown, and thus the opportunity to glean data from the 10 ICEMR surveillance sites is highly valuable. Resistance can be assessed by clinical trials comparing antimalarial efficacies of different agents, ex vivo/in vitro assessment of sensitivities of cultured *P. falciparum*, evaluation of genetic polymorphisms associated with resistance, or by assessing the selective pressure of antimalarial treatment on subsequent infections. Studies considering all of these factors have shed light on the extent of resistance and on mechanisms of resistance.

Resistance mediated by transporter mutations. The *P. falciparum* genome encodes multiple predicted transporters.¹⁶ Polymorphisms in transport proteins can mediate resistance to many agents active against cancer and infectious diseases via enhancing efflux of the drugs from cells.¹⁷ It appears that a number of plasmodial proteins transport different drugs and that polymorphisms in these proteins may impact on drug sensitivity.¹⁸ *pfmdr1*. Polymorphisms in the *P. falciparum* multidrug resistance-1 (*pfmdr1*) gene, which encodes the P-glycoprotein

homolog, impact on sensitivity to multiple antimalarial drugs.^{19–21} In humans, P-glycoprotein polymorphisms are associated with resistance to cancer drugs.²² In *P. falciparum*, the function of the *pfmdr1* product is unknown, but the protein localizes to the membrane of the food vacuole, the site of action of a number of drugs, suggesting that it is a drug transporter.²³ Data on associations between *pfmdr1* polymorphisms and drug sensitivity are complex, but overall suggest that changes in *pfmdr1* sequence or copy number alter transport of multiple drugs in or out of the parasite food vacuole, with individual polymorphisms leading to opposite effects on different drugs.²⁴ Increased copy number of *pfmdr1*, which is prevalent in southeast Asia, has been associated with extensive use of mefloquine.²⁵ Experimental evidence indicates that *pfmdr1* amplification also leads to decreased sensitivity to quinine, lumefantrine, and artemisinin.²⁶ Mutations at *pfmdr1* N86Y and D1246Y (for this and other *P. falciparum* genes, wild type sequence is based on the 3D7 reference strain), which are common in Africa, have been linked to decreased sensitivity to chloroquine and amodiaquine, but increased sensitivity to lumefantrine, mefloquine, and artemisinins.^{27–31} Other polymorphisms primarily seen outside Africa (including 1034C and 1042D) are associated with altered sensitivity to lumefantrine, mefloquine, and artemisinins.^{26,29,32–34} Considering infections that emerge soon after prior therapy, amodiaquine-containing regimens selected for the 86Y and 1246Y mutant alleles^{35–37} and for parasites with decreased in vitro sensitivity to the active metabolite monodesethylamodiaquine³⁸ in subsequent infections. The selective pressure of the related aminoquinoline piperazine seems less marked than that of amodiaquine, but prior use of the drug also selected for the *pfmdr1* 86Y and 1246Y mutations.^{31,39} In contrast, therapy with artemether/lumefantrine selected for the N86 and D1246 wild type alleles in subsequent infections within 60 days of prior therapy.^{31,35,36,39–43} Importantly, impacts of *pfmdr1* polymorphisms on drug sensitivity are modest, correlations between particular polymorphisms and treatment efficacy have not been seen, and the ACTs artesunate/amodiaquine and artemether/lumefantrine remain highly efficacious for the treatment of uncomplicated falciparum malaria in Africa.^{44,45} However, as seen for chloroquine and amodiaquine, *pfmdr1* polymorphisms may contribute, with additional polymorphisms, to resistance to increasingly used components of ACTs.

pfcr1. Soon after the identification of *pfmdr1*, it became clear that polymorphisms in this gene are not the primary mediators of chloroquine resistance. Subsequently, analysis of progeny of a genetic cross between chloroquine sensitive and resistant strains led to the identification of *pfcr1*,⁴⁶ which encodes a food vacuole membrane protein that is predicted to be a member of the drug/metabolite transporter superfamily.^{47,48} The function of *pfcr1* is unknown, but apparently essential, as disruption of the gene has not been possible.⁴⁹ *pfcr1* is highly polymorphic, but one single nucleotide polymorphism (SNP), K76T, is the primary mediator of chloroquine resistance.^{49,50} The 76T mutation appears to act principally by increasing the export of chloroquine from the food vacuole, but the mechanism of *pfcr1* 76T-mediated chloroquine resistance is incompletely understood.⁴⁹ Other *pfcr1* SNPs always accompany 76T in field isolates, and these likely encode compensatory mutations that allow parasites containing 76T to maintain adequate fitness; some other SNPs may also contribute directly to the drug resistant phe-

notype. The 76T mutation also mediates decreased sensitivity to monodesethylamodiaquine, and studies with genetically modified parasites have shown it to mediate increased susceptibility to mefloquine and artemisinins,⁵¹ suggesting the same reciprocal relationship between sensitivities to aminoquinolines and other drugs as described for certain *pfmdr1* polymorphisms.

Pfmrp1. *Plasmodium falciparum* multidrug resistance protein-1 (*Pfmrp1*) is a member of the ABC transporter superfamily.²⁴ In studies of culture-adapted *P. falciparum*, SNPs in *pfmrp1* were linked to decreased sensitivity to chloroquine and quinine.¹⁶ Two SNPs that appear to be common in African parasites, I876V and K1466R, were selected by prior treatment with artemether/lumefantrine⁵² and sulfadoxine/pyrimethamine,⁵³ respectively, although these SNPs were not associated with altered drug sensitivity in African isolates.³¹ *pfmrp1* mutations appear to differ between continents; some SNPs in northeast Myanmar isolates were associated with reduced susceptibilities to chloroquine, mefloquine, pyronaridine, and lumefantrine.⁵⁴ Disruption of the *pfmrp1* gene yielded parasites with diminished growth and increased sensitivity to chloroquine and other drugs, suggesting a role for this protein in the efflux of antimalarial drugs from the parasite and in parasite fitness.⁵⁵

Sodium transporters. Quantitative trait locus analysis identified three genes predicted to play roles in the responsiveness of *P. falciparum* to quinine, *pfcr1*, *pfmdr1*, and *pfnhel1*, which encode a putative sodium-hydrogen exchanger and are highly polymorphic.⁵⁶ *pfatp4* encodes a *P. falciparum* plasma membrane protein that appears to be a sodium efflux pump.⁵⁷ Recent studies have shown that three different classes of potent antimalarial compounds, spiroindolones, pyrazoleamides, and dihydroisoquinolones, all target *pfatp4*.^{57–59} Mutations in *pfatp4* have been linked to altered sensitivity to these candidate antimalarials.^{58–60} In addition, screening of the “Malaria Box” chemical library identified 28 compounds of diverse chemotypes that affected parasite Na⁺ and pH regulation in a manner consistent with PfATP4 inhibition.⁶¹ A recent clinical trial of the spiroindolone KAE609 demonstrated excellent efficacy against falciparum and vivax malaria.⁶²

Resistance to quinine. Resistance to quinine, the oldest antimalarial drug, was reported first in Brazil⁶³ and later in southeast Asia.^{64,65} Quinine resistance is associated with polymorphisms in several transporters. As stated earlier, SNPs in *pfmdr1*, *pfcr1*, and *pfmrp1* are linked to decreased sensitivity to quinine. In addition, *pfmdr1* gene amplification can also lead to quinine resistance.⁶⁶ Recent studies evaluating associations between polymorphisms in a *pfnhel1* microsatellite, in vitro parasite sensitivity, and clinical responses to various drugs have been inconsistent, but these polymorphisms appear to have a modest impact on sensitivity of parasites to quinine, and possibly other drugs.^{67–72} In vitro allelic exchange to reduce the expression of *pfnhel1* by ~50% led to a 30% increase in quinine sensitivity in some, but not other parasite strains.⁷³

Resistance to antifolates. The parasite-specific antimetabolite, pyrimethamine, is usually discussed in combination with its partner drug sulfadoxine (known as SP or Fansidar). Pyrimethamine was first used as an individual drug, but resistance was seen within a year in both *P. vivax* and *P. falciparum*.^{74,75} The combination of sulfa drugs and pyrimethamine proved to be potent in the laboratory, as well as in the field against chloroquine-resistant uncomplicated malaria but, again, resistance appeared rapidly in the Asia Pacific regions in the late 1970s, as well as in South America.^{76–79}

Molecular genetic studies attribute pyrimethamine and sulfa resistance to mutations in the genes coding for the target enzymes DHFR and DHPS.^{80,81} These markers have been useful for tracking sulfadoxine/pyrimethamine resistance across the globe, and show particular promise with new multiplex strategies.⁸² In the 1990s, sulfadoxine/pyrimethamine found increasing use in Africa to treat widespread chloroquine-resistant malaria, before sulfadoxine/pyrimethamine resistance followed. Molecular epidemiology studies utilizing DNA microsatellite sequences flanking the *dhfr* gene point to transfer of pyrimethamine resistance from Asia to Africa, possibly from a single ancestor and possibly before sulfadoxine/pyrimethamine use even began in Africa.⁸³ In contrast, resistance to sulfa partners through *dhps* mutations seemed to occur through de novo events both in sub-Saharan Africa and in Asia.^{84,85} Sulfadoxine/pyrimethamine is no longer recommended as a first-line drug for the treatment of *P. falciparum*. However, it continues to be used in ACT combinations in most parts of India,⁸⁶ for intermittent preventive therapy in pregnant women in Africa,⁸⁷ and for seasonal malaria chemoprevention in children in the sub-Sahel of Africa,⁸⁸ although widespread resistance limits these interventions.^{89,90} Perhaps not surprisingly, sulfadoxine/pyrimethamine resistance in *P. vivax* appeared in Asia and the Pacific Islands, where *P. falciparum* and *P. vivax* coexist.^{91–94}

Resistance to artemisinin family drugs. Since artemisinins play an indispensable role in current malaria therapies, artemisinin resistance has received wide recent attention. In the Cambodia–Thailand border region of southeast Asia, an epicenter of antimalarial drug resistance, declining efficacy of the artesunate/mefloquine combination was noted,⁹⁵ and clinical resistance to artesunate, manifested as delayed clearance of parasitemia after therapy, but not generally as full-blown treatment failure, was documented in 2008.^{96–98} The delayed parasite clearance phenotype does not correspond to increased artemisinin half maximal inhibitory concentration (IC₅₀) values, as determined by standard in vitro assays, but does correspond to decreased susceptibility assessed 72 hours after a pulse of dihydroartemisinin using the new ring-stage survival assay.^{99,100} Attempts to select resistance to artemisinins in vitro using constant or pulsed drug selection pressure initially identified *pfmdr1* amplification and increased antioxidant levels in selected parasites.^{101,102} In field-based studies, genome-wide association studies identified regions on chromosome 13 linked to delayed parasite clearance.^{103,104} Using a combined resistance selection and genomic approach, Ariey and others¹⁰⁵ identified mutations in the propeller domain of the *P. falciparum* kelch (*K13*) gene (PF3D7_1343700) associated with delayed parasite clearance after artemisinin therapy in southeast Asia.

Very recently, using clinical and molecular data, the extent of artemisinin resistance has been delineated, with delayed clearance and *K13* mutations common in parts of Cambodia, Thailand, Myanmar, and Vietnam, but not in other areas of Asia or Africa.^{106–108} Other reports from Cambodia have shown recrudescence infections after treatment with dihydroartemisinin/piperazine, raising the concern that resistance to artemisinin partner drugs has been facilitated by the spread of artemisinin resistance.¹⁰⁹ In African parasites, although *K13* gene polymorphisms are common, including some mutations in the propeller domain, the specific mutations associated with artemisinin resistance in southeast Asia have not been seen.^{110–112} Parasites with introduced *K13* mutations showed enhanced survival after a dihydroartemisinin pulse, confirming the role of these mutations in resistance.¹¹³ The transcriptomes of resistant parasites showed increased expression of unfolded protein response pathways and prolonged ring-stage development, offering insights into the mechanism of artemisinin resistance.¹¹⁴

Resistance to Malarone. Atovaquone is a potent inhibitor of electron transport, and studies identified the target of this drug as the critical quinone-binding sites of cytochrome b.^{115,116} When the drug is used alone, resistance develops rapidly and recrudescence after therapy is common. Resistance is conferred by single-point mutations in the cytochrome b (*Pficyt*) gene. *Pficyt* mutations 268S and 268N were associated with Malarone treatment failure.^{117,118} However, treatment failure has also been reported in the absence of these mutations.^{119–121}

ICEMR DATA CONCERNING ANTIMALARIAL DRUG RESISTANCE

Data from ICEMR sites, collected both before and during enactment of the ICEMR programs, offer insight into global drug resistance trends. Research activities at different ICEMR sites entail clinical studies, ex vivo/in vitro assays, and molecular studies (Table 2).

Clinical observations. Clinical trials in west Africa, Uganda, south Asia, and Papua New Guinea (PNG) have generally shown excellent antimalarial efficacy for the ACTs artemether/lumefantrine, artesunate/amodiaquine, and dihydroartemisinin/piperazine (Supplemental Table 1). In high transmission settings, new infections after ACT therapy may be common, but true recrudescences after treatment have been very uncommon. In India, particularly in the northeast along the Myanmar border, where artesunate/sulfadoxine–pyrimethamine combinations are being discontinued, there have been excellent

TABLE 2
Drug resistance surveillance activities in ICEMRs and ICEMR regions

| ICEMR | Drug efficacy trials | Parasite clearance data | Ex vivo drug sensitivity* | In vitro drug sensitivity† | Drug resistance polymorphisms |
|-----------------|----------------------|-------------------------|---------------------------|----------------------------|-------------------------------|
| West Africa | Yes | Yes | Yes | Yes | Yes |
| Southern Africa | No | Yes | No | No | Yes |
| Malawi | Yes | Yes | No | No | Yes |
| Uganda | Yes | Yes | Yes | Yes | Yes |
| South Asia | Yes‡ | Yes | No | Yes | Yes |
| Southeast Asia | Yes | Yes | No | Yes | Yes |
| PNG | Yes | No | Yes | No | No |

ICEMR = the International Centers of Excellence for Malaria Research; PNG = Papua New Guinea.

*Characterization of sensitivity in fresh samples from infected subjects.

†Characterization of sensitivity in culture-adapted parasites.

‡Conducted by ICEMR partners.

responses to artesunate/mefloquine, artesunate/amodiaquine, and dihydroartemisinin/piperaquine (Supplemental Table 1). Even in low-endemicity areas at the China–Myanmar border, where artemisinins have the longest history of use, excellent efficacy of the ACTs for treatment of falciparum malaria has been seen.¹²² On the other hand, an effectiveness study in Uganda showed a failure rate of 31% after treatment with quinine.¹²³ In regions of *P. falciparum*/*P. vivax* co-endemicity, *P. falciparum* typically shows rapid responses to control efforts, whereas *P. vivax* prevalence subsides more slowly. In northeast Myanmar, follow-ups of *P. vivax* cases after chloroquine/primaquine treatment indicated an increase in cases with recurrent parasitemia within 28 days compared with a prior report,¹²⁴ suggesting the emergence of chloroquine resistance.¹²⁵ Ongoing clinical efficacy studies at the ICEMR sentinel sites will be important to offer a longitudinal appreciation of drug efficacy and provide a scientific basis to guide local drug policy (Table 3).

Ex vivo and in vitro studies. Studies on ex vivo (parasites studied immediately after collection from infected patients) or in vitro (parasites studied after culture adaptation) anti-malarial drug sensitivity of *P. falciparum* have been conducted by ICEMR groups from west Africa, Uganda, south Asia, southeast Asia, and PNG (Supplemental Table 2). Ex vivo studies have the advantage of testing samples directly from patients without potential selection biases due to constraints of culturing and cryopreservation. However, the results may be confounded by the presence of multiple clones of parasites that differ in sensitivities to the test drugs. In vitro assays performed with culture-adapted parasite clones allow assays to include multiple biological replications and provide better opportunities for subsequent genetic analysis. In general, these studies have shown that African parasites have varied sensitivities to chloroquine and amodiaquine, and good sensitivities to dihydroartemisinin, the active metabolite of all artemisinin derivatives, and to the ACT partner drugs lumefantrine, mefloquine, and piperaquine.^{31,126,127} In Uganda, increased deployment of artemether/lumefantrine was linked to some decrease in in vitro susceptibility to lumefantrine.³¹ In Thailand, reduced lumefantrine susceptibility might have resulted from extensive use of mefloquine, another amino-alcohol.¹²⁸ Clinical and in vitro resistance to quinine has been seen in southeast Asia, but not consistently in Africa. In Senegal and Uganda, for example, *P. falciparum* parasites appeared to be susceptible to quinine in vitro.^{126,129} In comparison, data from southeast Asia showed a mean IC₅₀ greater than 500 nM.⁶⁸ Similar to African parasites, southeast Asian isolates were generally sensitive to artemisinins and the ACT partner drugs lumefantrine and mefloquine.¹³⁰ Yet, longitudinal studies revealed gradual decrease of susceptibility to piperaquine, and a high correlation between chloroquine and piperaquine IC₅₀ values.¹³¹ However, only limited results are available, and considerations of ex vivo/in vitro results is com-

plicated by varied methodologies used by different groups, difficulties of interpreting results for polyclonal infections, and uncertain correlations between in vitro findings and clinical efficacy. Commonly used ex vivo/in vitro assays measure the parasite histidine-rich protein-2 by enzyme-linked immunosorbent assay, replication of parasite DNA by isotope incorporation, or use of a fluorescent dye such as SBYR Green I.^{132,133}

To enhance comparisons among sites, ex vivo/in vitro assays should consider the inclusion of a standard laboratory strain (such as 3D7) as an internal control. Further, the new ring-stage survival assay⁹⁹ should be adopted in multiple ICEMR sites to monitor the emergence and spread of artemisinin resistance. Some ICEMRs also have prevalent transmission of vivax malaria. Ex vivo drug assays for *P. vivax* are also being conducted (Table 3), but the assays are constrained by difficulties of *P. vivax* culture and the appreciation that assays for certain drugs (e.g., chloroquine) require a high proportion of parasites at the ring stage and a high parasitemia.

Genotyping drug resistance–mediating polymorphisms. Studies of genetic polymorphisms associated with drug resistance are technically much simpler than in vitro studies of parasite sensitivity, and so results are more widely available. Studies of *P. falciparum* genetic polymorphisms have been conducted by ICEMR groups from west Africa, southern Africa, Uganda, south Asia, southeast Asia, and PNG (Supplemental Tables 3 and 4). As has already been well documented in past studies, the prevalence of a number of polymorphisms that impact on drug sensitivity varies greatly around the world. Also of interest are changes in polymorphism prevalence over time. In Uganda, parasites have demonstrated marked changes in the prevalence of some key polymorphisms over the last decade, coincident with changes in treatment practices for malaria from chloroquine to chloroquine/sulfadoxine–pyrimethamine to artemether/lumefantrine. Most notably, the prevalence of three wild type alleles, *pfprt* K76, *pfmdr1* N86, and *pfmdr1* D1246, has all increased markedly in recent years¹³⁴ and this increase was greater in children treated with artemether/lumefantrine for all episodes of malaria than in those treated with dihydroartemisinin/piperaquine.³⁹ This is in sharp contrast to the *P. falciparum* parasites at the China–Myanmar border area, where *pfprt* 76T and 220S remained almost fixed in a recent study.¹³⁵ Recently, the Uganda ICEMR group showed that therapy with artemether/lumefantrine selects for the wild-type polymorphisms associated with decreased lumefantrine efficacy and, in ex vivo studies, for parasites with diminished lumefantrine sensitivity.³¹ Importantly, despite these changes, sensitivity to lumefantrine remains quite good, and artemether/lumefantrine treatment efficacy is excellent. However, recent unpublished trials (Yeka and others, unpublished data) showed that in 2013–2014 artemether/lumefantrine was less efficacious than artesunate/amodiaquine at three different sites in Uganda, a change in relative treatment efficacy compared with older findings.^{44,136} These results suggest that

TABLE 3
Studies of *Plasmodium vivax*

| ICEMR/region | Drug efficacy trials | | | Drug resistance polymorphisms | |
|----------------|------------------------|--------------------------------|--------------------------|-------------------------------|---------------|
| | ACT | CQ/primaquine | Ex vivo drug sensitivity | <i>pvmdr1</i> | <i>pvdhfr</i> |
| Southeast Asia | No | Yes | Yes | Yes | No |
| South Asia | Yes | Yes | Yes | Yes | Yes |
| PNG | AL, DP, ART/NQ, ART-SP | CQ-SP and AQ-SP, no primaquine | No | Yes | Yes |

ACT = artemisinin-based combination therapy; AL = artemether/lumefantrine; ART = artemisinin; AQ = amodiaquine; CQ = chloroquine; DP = dihydroartemisinin/piperaquine; ICEMR = the International Centers of Excellence for Malaria Research; MQ = mefloquine; NQ = naphthoquine; PNG = Papua New Guinea; SP = sulfadoxine/pyrimethamine.

recent changes in treatment practices have led to changes in *P. falciparum* in Uganda that have mediated decreased antimalarial efficacy of artemether/lumefantrine, the first-line antimalarial drug in the country. An urgent issue that the ICEMRs are addressing is monitoring of the emergence and/or spread of artemisinin resistance.¹¹⁰ The identification of the *K13* gene as a molecular marker for artemisinin resistance will facilitate large-scale surveillance.¹³⁷

RESISTANCE AND FITNESS

One of the challenges to studying the interplay between parasite drug resistance and fitness is the lack of a direct measure for fitness. Comparison of relative growth rates in vitro or ex vivo is the commonly used approach, although growth rates may not represent relative fitness in the natural host. Assessment of parasite survival in the field provides an improved measure, although analyses are challenging. It has long been observed that *P. falciparum* genetic mutations that confer drug resistance are associated with altered biological fitness of the parasite.^{138–140} However, various investigators have reported both increased^{141–143} and decreased^{144–146} fitness in resistant parasites. The latter would seem intuitive from initial low prevalence of innate resistance observed in the field for some antimalarials such as mefloquine and atovaquone, presumably owing to the mutant parasites being outcompeted by the wild type before start of drug use.^{117,147,148} More compelling evidence for a fitness cost of resistance has been documented by reemergence of sensitive parasites, virtually replacing highly prevalent resistant strains, following withdrawal of drug (chloroquine or sulfadoxine–pyrimethamine) pressure in the population.^{149–154} The rub is that in other areas under similar conditions, reemergence has occurred much more slowly or not at all.^{155–159} Similarly cogent evidence of a fitness cost to resistance has been demonstrated by decreased prevalence of resistant parasites after the dry season in west Africa, when there is relatively little drug selection pressure.^{160–162} Again, other studies have had contrasting results.^{163,164}

Global data from the ICEMRs afford an opportunity for a concerted evidence base on associations between resistance and fitness, and potentially on the de facto risk factors for the emergence or suppression of drug resistance in the field. So far drug selection pressure, herd immunity,^{153,160} and ecological differences^{146,165,166} have been shown to impinge on relative fitness of drug-resistant and drug-sensitive parasites in the wild. Although combination of antimalarial compounds with opposing resistance mechanisms have been used to suppress the emergence of drug resistance in laboratory isolates,¹⁶⁷ opposite resistance selection has also been observed in the field between 4-aminoquinolines (chloroquine, amodiaquine) and artemisinins.^{27,168–170} Data from Uganda showed significantly lower prevalence of symptoms among children infected with parasites containing chloroquine resistance mutations compared with those infected with wild-type parasites, consistent with greater virulence for wild-type parasites.¹⁷¹ Field data from the southern Africa ICEMR suggest a role for the vector in selecting drug resistance polymorphisms, with significant differences in prevalence of SNPs that mediate resistance to aminoquinolines and antifolates between parasites infecting mosquitoes and people.^{172,173} So far, *Anopheles arabiensis*^{172,173} and more recently *Anopheles funestus* (Matsena and others, unpublished

data) have both been shown to exert selection on drug resistance polymorphisms. This would seem to explain the role of ecology in governing resistance,^{165,166} which can differ between different regions of the same country. The unexpected link between vector control and prevalence of drug-resistant malaria parasites in some areas^{174–177} but not others¹⁷⁸ also seems consistent with vector selection. More detailed data from multiple ICEMRs will be instrumental in improving our understanding of the interplay between drug resistance and fitness, and hopefully the development of more effective strategies for the containment of drug-resistant malaria.

CONCLUSION AND FUTURE STUDIES

In an evolutionary arms race between malaria parasites and a series of therapeutic interventions, the parasites have consistently been able to develop resistance to each new class of drugs. The emergence of parasites resistant to artemisinins in southeast Asia and altered sensitivities to artemisinin partner drugs pose great threats to efforts to control and, eventually, eradicate malaria. Specifically, previous failures of the ACTs artesunate/mefloquine and artesunate/amodiaquine have recently been followed by frequent failures of dihydroartemisinin/piperazine in parts of Cambodia, and decreasing sensitivity to lumefantrine may further threaten artemether/lumefantrine. It is thus of high priority to continue surveillance of ACT efficacy, the ex vivo and in vitro activities of ACT components, and molecular markers that may mediate resistance to these drugs. For artemisinins, mutations in the *K13* gene offer markers for the delayed parasite clearance phenotype that is now common in parts of southeast Asia. Mutations in the putative drug transporters *pfmdr1* and *pfert* mediate altered sensitivity to multiple artemisinin partner drugs, including amodiaquine, mefloquine, lumefantrine, and piperazine, although different drugs are impacted in opposite directions. Additional parasite polymorphisms are likely important in drug responsiveness, and an improved understanding of the roles of these polymorphisms is an important goal. Additional studies on the influence of drug resistance on parasite fitness may enable the identification of optimal dosing strategies, including, possibly, rotating of regimens. Further, understanding how mosquitoes mediate the spread of drug resistance and use of evolution-proof mosquito control measures may help to deter resistance spread, enabling the regional elimination and eventual eradication of malaria.

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REFERENCES

- Murray CJ, Rosenfeld LC, Lim SS, Andrews KG, Foreman KJ, Haring D, Fullman N, Naghavi M, Lozano R, Lopez AD, 2012. Global malaria mortality between 1980 and 2010: a systematic analysis. *Lancet* 379: 413–431.
- Tjitra E, Anstey NM, Sugiarto P, Warikar N, Kenangalem E, Karyana M, Lampah DA, Price RN, 2008. Multidrug-resistant *Plasmodium vivax* associated with severe and fatal malaria: a prospective study in Papua, Indonesia. *PLoS Med* 5: e128.
- Okumu FO, Moore SJ, 2011. Combining indoor residual spraying and insecticide-treated nets for malaria control in Africa: a review of possible outcomes and an outline of suggestions for the future. *Malar J* 10: 208.
- Olotu A, Fegan G, Wambua J, Nyangweso G, Awuondo KO, Leach A, Lievens M, Lebouilleux D, Njuguna P, Peshu N, Marsh K, Bejon P, 2013. Four-year efficacy of RTS,S/AS01E and its interaction with malaria exposure. *N Engl J Med* 368: 1111–1120.
- Gosling RD, Okell L, Moshia J, Chandramohan D, 2011. The role of antimalarial treatment in the elimination of malaria. *Clin Microbiol Infect* 17: 1617–1623.
- Greenwood B, 2010. Anti-malarial drugs and the prevention of malaria in the population of malaria endemic areas. *Malar J* 9 (Suppl 3): S2.
- Nosten F, White NJ, 2007. Artemisinin-based combination treatment of falciparum malaria. *Am J Trop Med Hyg* 77: 181–192.
- Schlagenhauf P, Petersen E, 2008. Malaria chemoprophylaxis: strategies for risk groups. *Clin Microbiol Rev* 21: 466–472.
- Baird JK, 2011. Resistance to chloroquine unhinges vivax malaria therapeutics. *Antimicrob Agents Chemother* 55: 1827–1830.
- Davis TM, Hung TY, Sim IK, Karunajeewa HA, Ilett KF, 2005. Piperaquine: a resurgent antimalarial drug. *Drugs* 65: 75–87.
- Meshnick SR, Dobson MJ, 2001. The history of antimalarial drugs. Rosenthal PJ, ed. *Antimalarial Chemotherapy: Mechanisms of Action, Resistance, and New Directions in Drug Discovery*. Totowa, NJ: Humana Press, 15–25.
- Dahl EL, Rosenthal PJ, 2008. Apicoplast translation, transcription and genome replication: targets for antimalarial antibiotics. *Trends Parasitol* 24: 279–284.
- Cui L, Su XZ, 2009. Discovery, mechanisms of action and combination therapy of artemisinin. *Expert Rev Anti Infect Ther* 7: 999–1013.
- Dondorp A, Nosten F, Stepniewska K, Day N, White N, 2005. Artesunate versus quinine for treatment of severe falciparum malaria: a randomised trial. *Lancet* 366: 717–725.
- Dondorp AM, Fanello CI, Hendriksen IC, Gomes E, Seni A, Chhaganlal KD, Bojang K, Olaosebikan R, Anunobi N, Maitland K, Kivaya E, Agbenyega T, Nguah SB, Evans J, Gesase S, Kahabuka C, Mtove G, Nadjm B, Deen J, Mwanga-Amumpaire J, Nansumba M, Karema C, Umulisa N, Uwimana A, Mokuolu OA, Adedoyin OT, Johnson WB, Tshetu AK, Onyamboko MA, Sakulthaew T, Ngum WP, Silamut K, Stepniewska K, Woodrow CJ, Bethell D, Wills B, Oneko M, Peto TE, von Seidlein L, Day NP, White NJ, 2010. Artesunate versus quinine in the treatment of severe falciparum malaria in African children (AQUAMAT): an open-label, randomised trial. *Lancet* 376: 1647–1657.
- Mu J, Ferdig MT, Feng X, Joy DA, Duan J, Furuya T, Subramanian G, Aravind L, Cooper RA, Wootton JC, Xiong M, Su XZ, 2003. Multiple transporters associated with malaria parasite responses to chloroquine and quinine. *Mol Microbiol* 49: 977–989.
- Borges-Walmsley MI, McKeegan KS, Walmsley AR, 2003. Structure and function of efflux pumps that confer resistance to drugs. *Biochem J* 376: 313–338.
- Picot S, Olliaro P, de Monbrison F, Bienvenu AL, Price RN, Ringwald P, 2009. A systematic review and meta-analysis of evidence for correlation between molecular markers of parasite resistance and treatment outcome in falciparum malaria. *Malar J* 8: 89.
- Foot SJ, Kyle DE, Martin RK, Oduola AM, Forsyth K, Kemp DJ, Cowman AF, 1990. Several alleles of the multidrug-resistance gene are closely linked to chloroquine resistance in *Plasmodium falciparum*. *Nature* 345: 255–258.
- Sanchez CP, Dave A, Stein WD, Lanzer M, 2010. Transporters as mediators of drug resistance in *Plasmodium falciparum*. *Int J Parasitol* 40: 1109–1118.
- Valderramos SG, Fidock DA, 2006. Transporters involved in resistance to antimalarial drugs. *Trends Pharmacol Sci* 27: 594–601.
- Sharom FJ, 2011. The P-glycoprotein multidrug transporter. *Essays Biochem* 50: 161–178.
- Cowman AF, Karcz S, Galatis D, Culvenor JG, 1991. A P-glycoprotein homologue of *Plasmodium falciparum* is localized on the digestive vacuole. *J Cell Biol* 113: 1033–1042.
- Koenderink JB, Kavishe RA, Rijpma SR, Russel FG, 2010. The ABCs of multidrug resistance in malaria. *Trends Parasitol* 26: 440–446.
- Price RN, Uhlemann AC, Brockman A, McGready R, Ashley E, Phaipun L, Patel R, Laing K, Looareesuwan S, White NJ, Nosten F, Krishna S, 2004. Mefloquine resistance in *Plasmodium falciparum* and increased *pfmdr1* gene copy number. *Lancet* 364: 438–447.
- Sidhu AB, Uhlemann AC, Valderramos SG, Valderramos JC, Krishna S, Fidock DA, 2006. Decreasing *pfmdr1* copy number in *Plasmodium falciparum* malaria heightens susceptibility to mefloquine, lumefantrine, halofantrine, quinine, and artemisinin. *J Infect Dis* 194: 528–535.
- Duraisingh MT, Jones P, Sambou I, von Seidlein L, Pinder M, Warhurst DC, 2000. The tyrosine-86 allele of the *pfmdr1* gene of *Plasmodium falciparum* is associated with increased sensitivity to the anti-malarials mefloquine and artemisinin. *Mol Biochem Parasitol* 108: 13–23.
- Duraisingh MT, Roper C, Walliker D, Warhurst DC, 2000. Increased sensitivity to the antimalarials mefloquine and artemisinin is conferred by mutations in the *pfmdr1* gene of *Plasmodium falciparum*. *Mol Microbiol* 36: 955–961.
- Reed MB, Saliba KJ, Caruana SR, Kirk K, Cowman AF, 2000. Pgh1 modulates sensitivity and resistance to multiple antimalarials in *Plasmodium falciparum*. *Nature* 403: 906–909.
- Mwai L, Kiara SM, Abdurahman A, Pole L, Rippert A, Diriye A, Bull P, Marsh K, Borrmann S, Nzila A, 2009. *In vitro* activity of piperaquine, lumefantrine and dihydroartemisinin in Kenyan *Plasmodium falciparum* isolates and polymorphisms in *Pfprt* and *Pfmdr1*. *Antimicrob Agents Chemother* 55: 5069–5073.
- Tumwebaze P, Conrad MD, Walakira A, LeClair N, Byaruhanga O, Nakazibwe C, Kozak B, Bloome J, Okiring J, Kakuru A, Bigira V, Kapisi J, Legac J, Gut J, Cooper RA, Kamya MR, Havlir DV, Dorsey G, Greenhouse B, Nsoyba SL, Rosenthal PJ, 2015. Impact of antimalarial treatment and chemoprevention on the drug sensitivity of malaria parasites isolated from Ugandan children. *Antimicrob Agents Chemother* 59: 3018–3030.
- Pickard AL, Wongsrichanalai C, Purfield A, Kamwendo D, Emery K, Zalewski C, Kawamoto F, Miller RS, Meshnick SR, 2003. Resistance to antimalarials in southeast Asia and genetic polymorphisms in *pfmdr1*. *Antimicrob Agents Chemother* 47: 2418–2423.
- Sidhu AB, Valderramos SG, Fidock DA, 2005. *pfmdr1* mutations contribute to quinine resistance and enhance mefloquine and artemisinin sensitivity in *Plasmodium falciparum*. *Mol Microbiol* 57: 913–926.
- Veiga MI, Ferreira PE, Jornhagen L, Malmberg M, Kone A, Schmidt BA, Petzold M, Bjorkman A, Nosten F, Gil JP, 2011. Novel polymorphisms in *Plasmodium falciparum* ABC transporter genes are associated with major ACT antimalarial drug resistance. *PLoS One* 6: e20212.

35. Humphreys GS, Merinopoulos I, Ahmed J, Whitty CJ, Mutabingwa TK, Sutherland CJ, Hallett RL, 2007. Amodiaquine and artemether-lumefantrine select distinct alleles of the *Plasmodium falciparum mdr1* gene in Tanzanian children treated for uncomplicated malaria. *Antimicrob Agents Chemother* 51: 991–997.
36. Zongo I, Dorsey G, Rouamba N, Tinto H, Dokomajilar C, Guiguemde RT, Rosenthal PJ, Ouedraogo JB, 2007. Artemether-lumefantrine versus amodiaquine plus sulfadoxine-pyrimethamine for uncomplicated falciparum malaria in Burkina Faso: a randomised non-inferiority trial. *Lancet* 369: 491–498.
37. Nsobya SL, Dokomajilar C, Joloba M, Dorsey G, Rosenthal PJ, 2007. Resistance-mediating *Plasmodium falciparum pfcr* and *pfmdr1* alleles after treatment with artesunate-amodiaquine in Uganda. *Antimicrob Agents Chemother* 51: 3023–3025.
38. 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* 200: 1650–1657.
39. Conrad MD, LeClair N, Arinaitwe E, Wanzira H, Kakuru A, Bigira V, Muhindo M, Kanya MR, Tappero JW, Greenhouse B, Dorsey G, Rosenthal PJ, 2014. Comparative impacts over 5 years of artemisinin-based combination therapies on *Plasmodium falciparum* polymorphisms that modulate drug sensitivity in Ugandan children. *J Infect Dis* 210: 344–353.
40. Sisowath C, Stromberg J, Martensson A, Msellem M, Obondo C, Bjorkman A, Gil JP, 2005. In vivo selection of *Plasmodium falciparum pfmdr1* 86N coding alleles by artemether-lumefantrine (Coartem). *J Infect Dis* 191: 1014–1017.
41. Happi CT, Gbotosho GO, Folarin OA, Sowunmi A, Hudson T, O'Neil M, Milhous W, Wirth DF, Oduola AM, 2009. Selection of *Plasmodium falciparum* multidrug resistance gene 1 alleles in asexual stages and gametocytes by artemether-lumefantrine in Nigerian children with uncomplicated falciparum malaria. *Antimicrob Agents Chemother* 53: 888–895.
42. Some AF, Sere YY, Dokomajilar C, Zongo I, Rouamba N, Greenhouse B, Ouedraogo JB, Rosenthal PJ, 2010. Selection of known *Plasmodium falciparum* resistance-mediating polymorphisms by artemether-lumefantrine and amodiaquine-sulfadoxine-pyrimethamine but not dihydroartemisinin-piperazine in Burkina Faso. *Antimicrob Agents Chemother* 54: 1949–1954.
43. Baliraine FN, Rosenthal PJ, 2011. Prolonged selection of *pfmdr1* polymorphisms after treatment of falciparum malaria with artemether-lumefantrine in Uganda. *J Infect Dis* 204: 1120–1124.
44. Dorsey G, Staedke S, Clark TD, Njama-Meya D, Nzarubara B, Maiteki-Sebuguzi C, Dokomajilar C, Kanya MR, Rosenthal PJ, 2007. Combination therapy for uncomplicated falciparum malaria in Ugandan children: a randomized trial. *JAMA* 297: 2210–2219.
45. Four Artemisinin-Based Combinations Study Group, 2011. A head-to-head comparison of four artemisinin-based combinations for treating uncomplicated malaria in African children: a randomized trial. *PLoS Med* 8: e1001119.
46. Fidock DA, Nomura T, Talley AK, Cooper RA, Dzekunov SM, Ferdig MT, Ursos LM, Sidhu AB, Naude B, Deitsch KW, Su XZ, Wootton JC, Roepe PD, Welles TE, 2000. Mutations in the *P. falciparum* digestive vacuole transmembrane protein PfCRT and evidence for their role in chloroquine resistance. *Mol Cell* 6: 861–871.
47. Martin RE, Kirk K, 2004. The malaria parasite's chloroquine resistance transporter is a member of the drug/metabolite transporter superfamily. *Mol Biol Evol* 21: 1938–1949.
48. Tran CV, Saier MH Jr, 2004. The principal chloroquine resistance protein of *Plasmodium falciparum* is a member of the drug/metabolite transporter superfamily. *Microbiology* 150: 1–3.
49. Ecker A, Lehane AM, Clain J, Fidock DA, 2012. PfCRT and its role in antimalarial drug resistance. *Trends Parasitol* 28: 504–514.
50. Lakshmanan V, Bray PG, Verdier-Pinard D, Johnson DJ, Horrocks P, Muhle RA, Alakpa GE, Hughes RH, Ward SA, Krogstad DJ, Sidhu AB, Fidock DA, 2005. A critical role for PfCRT K76T in *Plasmodium falciparum* verapamil-reversible chloroquine resistance. *EMBO J* 24: 2294–2305.
51. Sidhu AB, Verdier-Pinard D, Fidock DA, 2002. Chloroquine resistance in *Plasmodium falciparum* malaria parasites conferred by *pfcr* mutations. *Science* 298: 210–213.
52. Dahlstrom S, Ferreira PE, Veiga MI, Sedighi N, Wiklund L, Martensson A, Farnert A, Sisowath C, Osorio L, Darban H, Andersson B, Kaneko A, Conseil G, Bjorkman A, Gil JP, 2009. *Plasmodium falciparum* multidrug resistance protein 1 and artemisinin-based combination therapy in Africa. *J Infect Dis* 200: 1456–1464.
53. Dahlstrom S, Veiga MI, Martensson A, Bjorkman A, Gil JP, 2009. Polymorphism in PfMRP1 (*Plasmodium falciparum* multidrug resistance protein 1) amino acid 1466 associated with resistance to sulfadoxine-pyrimethamine treatment. *Antimicrob Agents Chemother* 53: 2553–2556.
54. Gupta B, Xu S, Wang Z, Sun L, Miao J, Cui L, Yang Z, 2014. *Plasmodium falciparum* multidrug resistance protein 1 (*pfmrp1*) gene and its association with in vitro drug susceptibility of parasite isolates from north-east Myanmar. *J Antimicrob Chemother* 69: 2110–2117.
55. Raj DK, Mu J, Jiang H, Kabat J, Singh S, Sullivan M, Fay MP, McCutchan TF, Su XZ, 2009. Disruption of a *Plasmodium falciparum* multidrug resistance-associated protein (PfMRP) alters its fitness and transport of antimalarial drugs and glutathione. *J Biol Chem* 284: 7687–7696.
56. Ferdig MT, Cooper RA, Mu J, Deng B, Joy DA, Su XZ, Welles TE, 2004. Dissecting the loci of low-level quinine resistance in malaria parasites. *Mol Microbiol* 52: 985–997.
57. Spillman NJ, Allen RJ, McNamara CW, Yeung BK, Winzeler EA, Diagona TT, Kirk K, 2013. Na⁺ regulation in the malaria parasite *Plasmodium falciparum* involves the cation ATPase PfATP4 and is a target of the spiroindolone antimalarials. *Cell Host Microbe* 13: 227–237.
58. Vaidya AB, Morrisey JM, Zhang Z, Das S, Daly TM, Otto TD, Spillman NJ, Wyratt M, Siegl P, Marfurt J, Wirjanata G, Sebayang BF, Price RN, Chatterjee A, Nagle A, Stasiak M, Charman SA, Angulo-Barturen I, Ferrer S, Belen Jimenez-Diaz M, Martinez MS, Gamo FJ, Avery VM, Ruecker A, Delves M, Kirk K, Berriman M, Kortagere S, Burrows J, Fan E, Bergman LW, 2014. Pyrazoleamide compounds are potent antimalarials that target Na⁺ homeostasis in intraerythrocytic *Plasmodium falciparum*. *Nat Commun* 5: 5521.
59. Jimenez-Diaz MB, Ebert D, Salinas Y, Pradhan A, Lehane AM, Myrand-Lapierre ME, O'Loughlin KG, Shackleford DM, Justino de Almeida M, Carrillo AK, Clark JA, Dennis AS, Diep J, Deng X, Duffy S, Endsley AN, Fedewa G, Guiguemde WA, Gomez MG, Holbrook G, Horst J, Kim CC, Liu J, Lee MC, Matheny A, Martinez MS, Miller G, Rodriguez-Alejandre A, Sanz L, Sigal M, Spillman NJ, Stein PD, Wang Z, Zhu F, Waterson D, Knapp S, Shelat A, Avery VM, Fidock DA, Gamo FJ, Charman SA, Mirsalis JC, Ma H, Ferrer S, Kirk K, Angulo-Barturen I, Kyle DE, DeRisi JL, Floyd DM, Guy RK, 2014. (+)-SJ733, a clinical candidate for malaria that acts through ATP4 to induce rapid host-mediated clearance of *Plasmodium*. *Proc Natl Acad Sci USA* 111: E5455–E5462.
60. Rottmann M, McNamara C, Yeung BK, Lee MC, Zou B, Russell B, Seitz P, Plouffe DM, Dharia NV, Tan J, Cohen SB, Spencer KR, Gonzalez-Paez GE, Lakshminarayana SB, Goh A, Suwanarusk R, Jegla T, Schmitt EK, Beck HP, Brun R, Nosten F, Renia L, Dartois V, Keller TH, Fidock DA, Winzeler EA, Diagona TT, 2010. Spiroindolones, a potent compound class for the treatment of malaria. *Science* 329: 1175–1180.
61. Lehane AM, Ridgway MC, Baker E, Kirk K, 2014. Diverse chemotypes disrupt ion homeostasis in the malaria parasite. *Mol Microbiol* 94: 327–339.
62. White NJ, Pukrittayakamee S, Phyo AP, Rueangweeraayut R, Nosten F, Jittamala P, Jeeyapant A, Jain JP, Lefevre G, Li R, Magnusson B, Diagona TT, Leong FJ, 2014. Spiroindolone KAE609 for falciparum and vivax malaria. *N Engl J Med* 371: 403–410.
63. da Silva AF, Benchimol JL, 2014. Malaria and quinine resistance: a medical and scientific issue between Brazil and Germany (1907–19). *Med Hist* 58: 1–26.
64. Giboda M, Denis MB, 1988. Response of Kampuchean strains of *Plasmodium falciparum* to antimalarials: in-vivo assessment

- of quinine and quinine plus tetracycline; multiple drug resistance *in vitro*. *J Trop Med Hyg* 91: 205–211.
65. Pukrittayakamee S, Supanaranond W, Looareesuwan S, Vanijanonta S, White NJ, 1994. Quinine in severe falciparum malaria: evidence of declining efficacy in Thailand. *Trans R Soc Trop Med Hyg* 88: 324–327.
 66. Cowman AF, Galatis D, Thompson JK, 1994. Selection for mefloquine resistance in *Plasmodium falciparum* is linked to amplification of the *pfmdr1* gene and cross-resistance to halofantrine and quinine. *Proc Natl Acad Sci USA* 91: 1143–1147.
 67. Henry M, Briolant S, Zettor A, Pelleau S, Baragatti M, Baret E, Mosnier J, Amalvict R, Fusai T, Rogier C, Pradines B, 2009. *Plasmodium falciparum* Na⁺/H⁺ exchanger 1 transporter is involved in reduced susceptibility to quinine. *Antimicrob Agents Chemother* 53: 1926–1930.
 68. Meng H, Zhang R, Yang H, Fan Q, Su X, Miao J, Cui L, Yang Z, 2010. *In vitro* sensitivity of *Plasmodium falciparum* clinical isolates from the China-Myanmar border area to quinine and association with polymorphism in the Na⁺/H⁺ exchanger. *Antimicrob Agents Chemother* 54: 4306–4313.
 69. Okombo J, Kiara SM, Rono J, Mwai L, Pole L, Ohuma E, Borrmann S, Ochola LI, Nzila A, 2010. *In vitro* activities of quinine and other antimalarials and *pfhe* polymorphisms in *Plasmodium* isolates from Kenya. *Antimicrob Agents Chemother* 54: 3302–3307.
 70. Andriantsoanirina V, Menard D, Rabearimanana S, Hubert V, Bouchier C, Tichit M, Bras JL, Durand R, 2010. Association of microsatellite variations of *Plasmodium falciparum* Na⁺/H⁺ exchanger (*Pfhe-1*) gene with reduced *in vitro* susceptibility to quinine: lack of confirmation in clinical isolates from Africa. *Am J Trop Med Hyg* 82: 782–787.
 71. Baliraine FN, Nsoby SL, Achan J, Tibenderana JK, Talisuna AO, Greenhouse B, Rosenthal PJ, 2011. Limited ability of *Plasmodium falciparum* *pfcr*, *pfmdr1*, and *pfhe1* polymorphisms to predict quinine *in vitro* sensitivity or clinical effectiveness in Uganda. *Antimicrob Agents Chemother* 55: 615–622.
 72. Sinou V, Quang le H, Pelleau S, Huong VN, Huong NT, Tai le M, Bertaux L, Desbordes M, Latour C, Long LQ, Thanh NX, Parzy D, 2011. Polymorphism of *Plasmodium falciparum* Na⁺/H⁺ exchanger is indicative of a low *in vitro* quinine susceptibility in isolates from Viet Nam. *Malar J* 10: 164.
 73. Nkrumah LJ, Riegelhaupt PM, Moura P, Johnson DJ, Patel J, Hayton K, Ferdig MT, Wellems TE, Akabas MH, Fidock DA, 2009. Probing the multifactorial basis of *Plasmodium falciparum* quinine resistance: evidence for a strain-specific contribution of the sodium-proton exchanger PfNHE. *Mol Biochem Parasitol* 165: 122–131.
 74. Hernandez T, Myatt AV, Coatney GR, Jeffery GM, 1953. Studies in human malaria. XXXIV. Acquired resistance to pyrimethamine (Daraprim) by the Chesson strain of *Plasmodium vivax*. *Am J Trop Med Hyg* 2: 797–804.
 75. Jones SA, 1953. Experiment to determine if a proguanil-resistant strain of *P. falciparum* would respond to large doses of pyrimethamine. *BMJ* 1: 977.
 76. Ferraroni JJ, Hayes J, 1979. Drug-resistant falciparum malaria among the Mayongong Indians in the Brazilian Amazon. *Am J Trop Med Hyg* 28: 909–911.
 77. Nurse GT, 1981. Fansidar-resistant falciparum malaria in Papua New Guinea. *Lancet* 1: 36–37.
 78. Rumans LW, Dennis DT, Atmosoedjono S, 1979. Fansidar resistant falciparum malaria in Indonesia. *Lancet* 2: 580–581.
 79. Hurwitz ES, Johnson D, Campbell CC, 1981. Resistance of *Plasmodium falciparum* malaria to sulfadoxine-pyrimethamine ('Fansidar') in a refugee camp in Thailand. *Lancet* 1: 1068–1070.
 80. Cowman AF, Morry MJ, Biggs BA, Cross GA, Foote SJ, 1988. Amino acid changes linked to pyrimethamine resistance in the dihydrofolate reductase-thymidylate synthase gene of *Plasmodium falciparum*. *Proc Natl Acad Sci USA* 85: 9109–9113.
 81. Peterson DS, Walliker D, Wellems TE, 1988. Evidence that a point mutation in dihydrofolate reductase-thymidylate synthase confers resistance to pyrimethamine in falciparum malaria. *Proc Natl Acad Sci USA* 85: 9114–9118.
 82. Nankoberanyi S, Mbogo GW, LeClair NP, Conrad MD, Tumwebaze P, Tukwasibwe S, Kamya MR, Tappero J, Nsoby SL, Rosenthal PJ, 2014. Validation of the ligase detection reaction fluorescent microsphere assay for the detection of *Plasmodium falciparum* resistance mediating polymorphisms in Uganda. *Malar J* 13: 95.
 83. Roper C, Pearce R, Nair S, Sharp B, Nosten F, Anderson T, 2004. Intercontinental spread of pyrimethamine-resistant malaria. *Science* 305: 1124.
 84. Lumb V, Das MK, Singh N, Dev V, Khan W, Sharma YD, 2011. Multiple origins of *Plasmodium falciparum* dihydropteroate synthetase mutant alleles associated with sulfadoxine resistance in India. *Antimicrob Agents Chemother* 55: 2813–2817.
 85. Alifrangis M, Nag S, Schousboe ML, Ishengoma D, Lusingu J, Pota H, Kavishe RA, Pearce R, Ord R, Lynch C, Dejene S, Cox J, Rwakimari J, Minja DT, Lemnge MM, Roper C, 2014. Independent origin of *Plasmodium falciparum* antifolate super-resistance, Uganda, Tanzania, and Ethiopia. *Emerg Infect Dis* 20: 1280–1286.
 86. Jain V, Basak S, Bhandari S, Bharti PK, Thomas T, Singh MP, Singh N, 2014. Burden of complicated malaria in a densely forested Bastar region of Chhattisgarh State (central India). *PLoS One* 9: e115266.
 87. ter Kuile FO, van Eijk AM, Filler SJ, 2007. Effect of sulfadoxine-pyrimethamine resistance on the efficacy of intermittent preventive therapy for malaria control during pregnancy: a systematic review. *JAMA* 297: 2603–2616.
 88. Konate AT, Yaro JB, Ouedraogo AZ, Diarra A, Gansane A, Soulama I, Kangoye DT, Kabore Y, Ouedraogo E, Ouedraogo A, Tiono AB, Ouedraogo IN, Chandramohan D, Cousens S, Milligan PJ, Sirima SB, Greenwood B, Diallo DA, 2011. Intermittent preventive treatment of malaria provides substantial protection against malaria in children already protected by an insecticide-treated bednet in Burkina Faso: a randomised, double-blind, placebo-controlled trial. *PLoS Med* 8: e1000408.
 89. Naidoo I, Roper C, 2013. Mapping 'partially resistant', 'fully resistant', and 'super resistant' malaria. *Trends Parasitol* 29: 505–515.
 90. Parikh S, Rosenthal PJ, 2010. Intermittent preventive therapy for malaria in pregnancy: is sulfadoxine-pyrimethamine the right drug? *Clin Pharmacol Ther* 87: 160–162.
 91. Miao M, Yang Z, Cui L, Ahlum J, Huang Y, Cui L, 2010. Different allele prevalence in the dihydrofolate reductase and dihydropteroate synthase genes in *Plasmodium vivax* populations from China. *Am J Trop Med Hyg* 83: 1206–1211.
 92. Auliff A, Wilson DW, Russell B, Gao Q, Chen N, Anh le N, Maguire J, Bell D, O'Neil MT, Cheng Q, 2006. Amino acid mutations in *Plasmodium vivax dhfr* and *dhps* from several geographical regions and susceptibility to antifolate drugs. *Am J Trop Med Hyg* 75: 617–621.
 93. Prajapati SK, Joshi H, Dev V, Dua VK, 2011. Molecular epidemiology of *Plasmodium vivax* anti-folate resistance in India. *Malar J* 10: 102.
 94. Ganguly S, Saha P, Chatterjee M, Maji AK, 2014. Prevalence of polymorphisms in antifolate drug resistance molecular marker genes *pvdhfr* and *pvdhps* in clinical isolates of *Plasmodium vivax* from Kolkata, India. *Antimicrob Agents Chemother* 58: 196–200.
 95. Wongsrichanalai C, Meshnick SR, 2008. Declining artesunate-mefloquine efficacy against falciparum malaria on the Cambodia-Thailand border. *Emerg Infect Dis* 14: 716–719.
 96. Noedl H, Se Y, Schaefer K, Smith BL, Socheat D, Fukuda MM, 2008. Evidence of artemisinin-resistant malaria in western Cambodia. *N Engl J Med* 359: 2619–2620.
 97. Dondorp AM, Nosten F, Yi P, Das D, Phyo AP, Tarning J, Lwin KM, Ariey F, Hanpithakpong W, Lee SJ, Ringwald P, Silamut K, Imwong M, Chotivanich K, Lim P, Herdman T, An SS, Yeung S, Singhasivanon P, Day NP, Lindegardh N, Socheat D, White NJ, 2009. Artemisinin resistance in *Plasmodium falciparum* malaria. *N Engl J Med* 361: 455–467.
 98. Phyo AP, Nkhoma S, Stepniewska K, Ashley EA, Nair S, McGready R, ler Moo C, Al-Saai S, Dondorp AM, Lwin KM, Singhasivanon P, Day NP, White NJ, Anderson TJ, Nosten F, 2012. Emergence of artemisinin-resistant malaria on the western border of Thailand: a longitudinal study. *Lancet* 379: 1960–1966.
 99. Witkowski B, Amaratunga C, Khim N, Sreng S, Chim P, Kim S, Lim P, Mao S, Sopha C, Sam B, Anderson JM, Duong S, Chhor CM, Taylor WR, Suon S, Mercereau-Puijalon O, Fairhurst RM,

- Menard D, 2013. 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* 13: 1043–1049.
100. Witkowski B, Khim N, Chim P, Kim S, Ke S, Kloeing N, Chy S, Duong S, Leang R, Ringwald P, Dondorp AM, Tripura R, Benoit-Vical F, Berry A, Gorgette O, Arieu F, Barale JC, Mercereau-Puijalon O, Menard D, 2013. Reduced artemisinin susceptibility of *Plasmodium falciparum* ring stages in western Cambodia. *Antimicrob Agents Chemother* 57: 914–923.
 101. Chen N, Chavchich M, Peters JM, Kyle DE, Gatton ML, Cheng Q, 2010. Deamplification of *pfmdr1*-containing amplicon on chromosome 5 in *Plasmodium falciparum* is associated with reduced resistance to artemisinin in vitro. *Antimicrob Agents Chemother* 54: 3395–3401.
 102. Cui L, Wang Z, Miao J, Miao M, Chandra R, Jiang H, Su XZ, Cui L, 2012. Mechanisms of in vitro resistance to dihydroartemisinin in *Plasmodium falciparum*. *Mol Microbiol* 86: 111–128.
 103. Cheeseman IH, Miller BA, Nair S, Nkhoma S, Tan A, Tan JC, Al Saai S, Phyo AP, Moo CL, Lwin KM, McGready R, Ashley E, Imwong M, Stepniewska K, Yi P, Dondorp AM, Mayxay M, Newton PN, White NJ, Nosten F, Ferdig MT, Anderson TJ, 2012. A major genome region underlying artemisinin resistance in malaria. *Science* 336: 79–82.
 104. Takala-Harrison S, Clark TG, Jacob CG, Cummings MP, Miotto O, Dondorp AM, Fukuda MM, Nosten F, Noedl H, Imwong M, Bethell D, Se Y, Lon C, Tynner SD, Saunders DL, Socheat D, Arieu F, Phyo AP, Starzengruber P, Fuehrer HP, Swoboda P, Stepniewska K, Flegg J, Arze C, Cerqueira GC, Silva JC, Ricklefs SM, Porcella SF, Stephens RM, Adams M, Kenefic LJ, Campino S, Auburn S, MacInnis B, Kwiatkowski DP, Su XZ, White NJ, Ringwald P, Plowe CV, 2013. Genetic loci associated with delayed clearance of *Plasmodium falciparum* following artemisinin treatment in southeast Asia. *Proc Natl Acad Sci USA* 110: 240–245.
 105. Arieu F, Witkowski B, Amaratunga C, Beghain J, Langlois AC, Khim N, Kim S, Duru V, Bouchier C, Ma L, Lim P, Leang R, Duong S, Sreng S, Suon S, Chhor CM, Bout DM, Menard S, Rogers WO, Genton B, Fandeur T, Miotto O, Ringwald P, Le Bras J, Berry A, Barale JC, Fairhurst RM, Benoit-Vical F, Mercereau-Puijalon O, Menard D, 2014. A molecular marker of artemisinin-resistant *Plasmodium falciparum* malaria. *Nature* 505: 50–55.
 106. Ashley EA, Dhorda M, Fairhurst RM, Amaratunga C, Lim P, Suon S, Sreng S, Anderson JM, Mao S, Sam B, Sopha C, Chhor CM, Nguon C, Sovannaroeth S, Pukrittayakamee S, Jittamala P, Chotivanich K, Chutasmit K, Suchatsoonthorn C, Runcharoen R, Hien TT, Thuy-Nhien NT, Thanh NV, Phu NH, Htut Y, Han KT, Aye KH, Mokuolu OA, Olaosebikan RR, Folaranmi OO, Mayxay M, Khanthavong M, Hongvanthong B, Newton PN, Onyamboko MA, Fanello CI, Tshetu AK, Mishra N, Valecha N, Phyo AP, Nosten F, Yi P, Tripura R, Borrmann S, Bashraheil M, Peshu J, Faiz MA, Ghose A, Hossain MA, Samad R, Rahman MR, Hasan MM, Islam A, Miotto O, Amato R, MacInnis B, Stalker J, Kwiatkowski DP, Bozdech Z, Jeeyapant A, Cheah PY, Sakulthaew T, Chalk J, Intharabut B, Silamut K, Lee SJ, Vihokhern B, Kunasol C, Imwong M, Tarning J, Taylor WJ, Yeung S, Woodrow CJ, Flegg JA, Das D, Smith J, Venkatesan M, Plowe CV, Stepniewska K, Guerin PJ, Dondorp AM, Day NP, White NJ, Tracking Resistance to Artemisinin Collaboration, 2014. Spread of artemisinin resistance in *Plasmodium falciparum* malaria. *N Engl J Med* 371: 411–423.
 107. Bosman P, Stassijns J, Nackers F, Canier L, Kim N, Khim S, Alipon SC, Chhor Char M, Chea N, Dysoley L, Van den Bergh R, Etienne W, De Smet M, Menard D, Kindermans JM, 2014. *Plasmodium* prevalence and artemisinin-resistant falciparum malaria in Preah Vihear Province, Cambodia: a cross-sectional population-based study. *Malar J* 13: 394.
 108. Tun KM, Imwong M, Lwin KM, Win AA, Hlaing TM, Hlaing T, Lin K, Kyaw MP, Plewes K, Faiz MA, Dhorda M, Cheah PY, Pukrittayakamee S, Ashley EA, Anderson TJ, Nair S, McDew-White M, Flegg JA, Grist EP, Guerin P, Maude RJ, Smithuis F, Dondorp AM, Day NP, Nosten F, White NJ, Woodrow CJ, 2015. Spread of artemisinin-resistant *Plasmodium falciparum* in Myanmar: a cross-sectional survey of the K13 molecular marker. *Lancet Infect Dis* 15: 415–421.
 109. Saunders DL, Vanachayangkul P, Lon C, 2014. Dihydroartemisinin-piperazine failure in Cambodia. *N Engl J Med* 371: 484–485.
 110. Conrad MD, Bigira V, Kapisi J, Muhindo M, Kanya MR, Havlir DV, Dorsey G, Rosenthal PJ, 2014. Polymorphisms in K13 and falcipain-2 associated with artemisinin resistance are not prevalent in *Plasmodium falciparum* isolated from Ugandan children. *PLoS One* 9: e105690.
 111. Kamau E, Campino S, Amenga-Etego L, Drury E, Ishengoma D, Johnson K, Mumba D, Kekre M, Yavo W, Mead D, Bouyou-Akotet M, Apinjoh T, Golassa L, Randrianarivojosia M, Andagalu B, Maiga-Ascofare O, Amambua-Ngwa A, Tindana P, Ghansah A, MacInnis B, Kwiatkowski D, Djimde AA, 2014. K13-propeller polymorphisms in *Plasmodium falciparum* parasites from sub-Saharan Africa. *J Infect Dis* 211: 1352–1355.
 112. Taylor SM, Parobek CM, DeConti DK, Kayentao K, Coulbaly SO, Greenwood BM, Tagbor H, Williams J, Bojang K, Njie F, Desai M, Kariuki S, Gutman J, Mathanga DP, Martensson A, Ngasala B, Conrad MD, Rosenthal PJ, Tshetu AK, Moormann AM, Vulule JM, Doumbo OK, Ter Kuile FO, Meshnick SR, Bailey JA, Juliano JJ, 2014. Absence of putative artemisinin resistance mutations among *Plasmodium falciparum* in sub-Saharan Africa: a molecular epidemiologic study. *J Infect Dis* 211: 680–688.
 113. Straimer J, Gnädig NF, Witkowski B, Amaratunga C, Duru V, Ramadani AP, Dacheux M, Khim N, Zhang L, Lam S, Gregory PD, Urnov FD, Mercereau-Puijalon O, Benoit-Vical F, Fairhurst RM, Menard D, Fidock DA, 2014. K13-propeller mutations confer artemisinin resistance in *Plasmodium falciparum* clinical isolates. *Science* 347: 428–431.
 114. Mok S, Ashley EA, Ferreira PE, Zhou L, Lin Z, Yeo T, Chotivanich K, Imwong M, Pukrittayakamee S, Dhorda M, Nguon C, Lim P, Amaratunga C, Suon S, Hien TT, Htut Y, Faiz MA, Onyamboko MA, Mayxay M, Newton PN, Tripura R, Woodrow CJ, Miotto O, Kwiatkowski DP, Nosten F, Day NP, Preiser PR, White NJ, Dondorp AM, Fairhurst RM, Bozdech Z, 2014. Population transcriptomics of human malaria parasites reveals the mechanism of artemisinin resistance. *Science* 347: 431–435.
 115. Vaidya AB, Lashgari MS, Pologe LG, Morrisey J, 1993. Structural features of *Plasmodium* cytochrome b that may underlie susceptibility to 8-aminoquinolines and hydroxynaphthoquinones. *Mol Biochem Parasitol* 58: 33–42.
 116. Srivastava IK, Rottenberg H, Vaidya AB, 1997. Atovaquone, a broad spectrum antiparasitic drug, collapses mitochondrial membrane potential in a malarial parasite. *J Biol Chem* 272: 3961–3966.
 117. Fivelman QL, Butcher GA, Adagu IS, Warhurst DC, Pasvol G, 2002. Malarone treatment failure and in vitro confirmation of resistance of *Plasmodium falciparum* isolate from Lagos, Nigeria. *Malar J* 1: 1.
 118. Korsinczky M, Chen N, Kotecka B, Saul A, Rieckmann K, Cheng Q, 2000. Mutations in *Plasmodium falciparum* cytochrome b that are associated with atovaquone resistance are located at a putative drug-binding site. *Antimicrob Agents Chemother* 44: 2100–2108.
 119. Wichmann O, Muehlen M, Gruss H, Mockenhaupt FP, Suttrop N, Jelinek T, 2004. Malarone treatment failure not associated with previously described mutations in the cytochrome b gene. *Malar J* 3: 14.
 120. Musset L, Bouchaud O, Matheron S, Massias L, Le Bras J, 2006. Clinical atovaquone-proguanil resistance of *Plasmodium falciparum* associated with cytochrome b codon 268 mutations. *Microbes Infect* 8: 2599–2604.
 121. Wichmann O, Muehlberger N, Jelinek T, Alifrangis M, Peyerl-Hoffmann G, Muhlen M, Grobusch MP, Gascon J, Matteelli A, Laferl H, Bisoffi Z, Ehrhardt Z, Cuadros J, Hatz C, Gjorup I, McWhinney P, Beran J, da Cunha S, Schulze M, Kollaritsch H, Kern P, Fry G, Richter J; European Network on Surveillance of Imported Infectious Diseases, 2004. Screening for mutations related to atovaquone/proguanil resistance in treatment failures and other imported isolates of *Plasmodium falciparum* in Europe. *J Infect Dis* 190: 1541–1546.

122. Wang Y, Yang Z, Yuan L, Zhou G, Lee M-C, Fan Q, Xiao Y, Cao Y, Yan G, Cui L, 2015. Clinical efficacy of dihydroartemisinin-piperaquine for the treatment of uncomplicated *Plasmodium falciparum* malaria at the China-Myanmar border. *Am J Trop Med Hyg* 93: 577–583.
123. Achan J, Tibenderana JK, Kyabayinze D, Wabwire Mangen F, Kanya MR, Dorsey G, D'Alessandro U, Rosenthal PJ, Talisuna AO, 2009. Effectiveness of quinine versus artemether-lumefantrine for treating uncomplicated falciparum malaria in Ugandan children: randomised trial. *BMJ* 339: b2763.
124. Liang GL, Sun XD, Wang J, Zhang ZX, 2009. Sensitivity of *Plasmodium vivax* to chloroquine in Laza City, Myanmar. *Chin J Parasitol Parasitic Dis* 27: 175–176.
125. Yuan L, Wang Y, Parker DM, Gupta B, Yang Z, Liu H, Fan Q, Cao Y, Xiao Y, Lee MC, Zhou G, Yan G, Baird JK, Cui L, 2015. Therapeutic responses of *Plasmodium vivax* malaria to chloroquine and primaquine treatment in northeastern Myanmar. *Antimicrob Agents Chemother* 59: 1230–1235.
126. Nsoby SL, Kiggundu M, Nanyunja S, Joloba M, Greenhouse B, Rosenthal PJ, 2010. In vitro sensitivities of *Plasmodium falciparum* to different antimalarial drugs in Uganda. *Antimicrob Agents Chemother* 54: 1200–1206.
127. Van Tyne D, Dieye B, Valim C, Daniels RF, Sene PD, Lukens AK, Ndiaye M, Bei AK, Ndiaye YD, Hamilton EJ, Ndir O, Mboup S, Volkman SK, Wirth DF, Ndiaye D, 2013. Changes in drug sensitivity and anti-malarial drug resistance mutations over time among *Plasmodium falciparum* parasites in Senegal. *Malar J* 12: 441.
128. Mungthin M, Khositnithikul R, Sithichot N, Suwandittakul N, Wattanaveeradej V, Ward SA, Na-Bangchang K, 2010. Association between the *pfmdr1* gene and in vitro artemether and lumefantrine sensitivity in Thai isolates of *Plasmodium falciparum*. *Am J Trop Med Hyg* 83: 1005–1009.
129. Ndiaye D, Patel V, Demas A, LeRoux M, Ndir O, Mboup S, Clardy J, Lakshmanan V, Daily JP, Wirth DF, 2010. A non-radioactive DAPI-based high-throughput in vitro assay to assess *Plasmodium falciparum* responsiveness to antimalarials—increased sensitivity of *P. falciparum* to chloroquine in Senegal. *Am J Trop Med Hyg* 82: 228–230.
130. Wang Z, Parker D, Meng H, Wu L, Li J, Zhao Z, Zhang R, Fan Q, Wang H, Cui L, Yang Z, 2012. In vitro sensitivity of *Plasmodium falciparum* from China-Myanmar border area to major ACT drugs and polymorphisms in potential target genes. *PLoS One* 7: e30927.
131. Hao M, Jia D, Li Q, He Y, Yuan L, Xu S, Chen K, Wu J, Shen L, Sun L, Zhao H, Yang Z, Cui L, 2013. In vitro sensitivities of *Plasmodium falciparum* isolates from the China-Myanmar border to piperaquine and association with polymorphisms in candidate genes. *Antimicrob Agents Chemother* 57: 1723–1729.
132. Noedl H, Wongsrichanalai C, Wernsdorfer WH, 2003. Malaria drug-sensitivity testing: new assays, new perspectives. *Trends Parasitol* 19: 175–181.
133. Abdul-Ghani R, Al-Maktari MT, Al-Shibani LA, Allam AF, 2014. A better resolution for integrating methods for monitoring *Plasmodium falciparum* resistance to antimalarial drugs. *Acta Trop* 137: 44–57.
134. Mbogo GW, Nankoberanyi S, Tukwasibwe S, Baliraine FN, Nsoby SL, Conrad MD, Arinaitwe E, Kanya M, Tappero J, Staedke SG, Dorsey G, Greenhouse B, Rosenthal PJ, 2014. Temporal changes in prevalence of molecular markers mediating antimalarial drug resistance in a high malaria transmission setting in Uganda. *Am J Trop Med Hyg* 91: 54–61.
135. Yang Z, Li C, Miao M, Zhang Z, Sun X, Meng H, Li J, Fan Q, Cui L, 2011. Multidrug-resistant genotypes of *Plasmodium falciparum*, Myanmar. *Emerg Infect Dis* 17: 498–501.
136. Bukirwa H, Yeka A, Kanya MR, Talisuna A, Banek K, Bakyaite N, Rwakimari JB, Rosenthal PJ, Wabwire-Mangen F, Dorsey G, Staedke SG, 2006. Artemisinin combination therapies for treatment of uncomplicated malaria in Uganda. *PLoS Clin Trials* 1: e7.
137. Ashley EA, Dhorda M, Fairhurst RM, Amaratunga C, Lim P, Suon S, Sreng S, Anderson JM, Mao S, Sam B, Sopha C, Chhor CM, Nguon C, Sovannaroeth S, Pukrittayakamee S, Jittamala P, Chotivanich K, Chutasmit K, Suchatsoonthorn C, Runcharoen R, Hien TT, Thuy-Nhien NT, Thanh NV, Phu NH, Htut Y, Han KT, Aye KH, Mokuolu OA, Olaosebikan RR, Folaranmi OO, Mayxay M, Khanthavong M, Hongvanthong B, Newton PN, Onyamboko MA, Fanello CI, Tshefu AK, Mishra N, Valecha N, Phyo AP, Nosten F, Yi P, Tripura R, Borrmann S, Bashraheil M, Peshu J, Faiz MA, Ghose A, Hossain MA, Samad R, Rahman MR, Hasan MM, Islam A, Miotto O, Amato R, MacInnis B, Stalker J, Kwiatkowski DP, Bozdech Z, Jeeyapant A, Cheah PY, Sakulthaew T, Chalk J, Intharabut B, Silamut K, Lee SJ, Vihokhern B, Kunasol C, Imwong M, Tarning J, Taylor WJ, Yeung S, Woodrow CJ, Flegg JA, Das D, Smith J, Venkatesan M, Plowe CV, Stepniewska K, Guerin PJ, Dondorp AM, Day NP, White NJ, 2014. Spread of artemisinin resistance in *Plasmodium falciparum* malaria. *N Engl J Med* 371: 411–423.
138. Felger I, Beck HP, 2008. Fitness costs of resistance to antimalarial drugs. *Trends Parasitol* 24: 331–333.
139. Hastings IM, Donnelly MJ, 2005. The impact of antimalarial drug resistance mutations on parasite fitness, and its implications for the evolution of resistance. *Drug Resist Updat* 8: 43–50.
140. Rosenthal PJ, 2013. The interplay between drug resistance and fitness in malaria parasites. *Mol Microbiol* 89: 1025–1038.
141. Marks F, Evans J, Meyer CG, Browne EN, Flessner C, von Kalckreuth V, Eggelte TA, Horstmann RD, May J, 2005. High prevalence of markers for sulfadoxine and pyrimethamine resistance in *Plasmodium falciparum* in the absence of drug pressure in the Ashanti region of Ghana. *Antimicrob Agents Chemother* 49: 1101–1105.
142. Brown KM, Costanzo MS, Xu W, Roy S, Lozovsky ER, Hartl DL, 2010. Compensatory mutations restore fitness during the evolution of dihydrofolate reductase. *Mol Biol Evol* 27: 2682–2690.
143. Sandefur CI, Wooden JM, Quaye IK, Sirawaraporn W, Sibley CH, 2007. Pyrimethamine-resistant dihydrofolate reductase enzymes of *Plasmodium falciparum* are not enzymatically compromised in vitro. *Mol Biochem Parasitol* 154: 1–5.
144. Abdel-Muhsin AM, Mackinnon MJ, Ali E, Nassir el-KA, Suleiman S, Ahmed S, Walliker D, Babiker HA, 2004. Evolution of drug-resistance genes in *Plasmodium falciparum* in an area of seasonal malaria transmission in eastern Sudan. *J Infect Dis* 189: 1239–1244.
145. Preechapornkul P, Imwong M, Chotivanich K, Pongtavornpinyo W, Dondorp AM, Day NP, White NJ, Pukrittayakamee S, 2009. *Plasmodium falciparum* *pfmdr1* amplification, mefloquine resistance, and parasite fitness. *Antimicrob Agents Chemother* 53: 1509–1515.
146. Temu EA, Kimani I, Tuno N, Kawada H, Minjas JN, Takagi M, 2006. Monitoring chloroquine resistance using *Plasmodium falciparum* parasites isolated from wild mosquitoes in Tanzania. *Am J Trop Med Hyg* 75: 1182–1187.
147. Oduola AM, Sowunmi A, Milhous WK, Kyle DE, Martin RK, Walker O, Salako LA, 1992. Innate resistance to new antimalarial drugs in *Plasmodium falciparum* from Nigeria. *Trans R Soc Trop Med Hyg* 86: 123–126.
148. WHO, 1984. *Advances in Malaria Chemotherapy*. Report of a WHO Scientific Group. Geneva, Switzerland: World Health Organization.
149. Hailemeskel E, Kassa M, Tadesse G, Mohammed H, Woyessa A, Tasew G, Sleshi M, Kebede A, Petros B, 2013. Prevalence of sulfadoxine-pyrimethamine resistance-associated mutations in *dhfr* and *dhps* genes of *Plasmodium falciparum* three years after SP withdrawal in Bahir Dar, northwest Ethiopia. *Acta Trop* 128: 636–641.
150. Ndiaye M, Faye B, Tine R, Ndiaye JL, Lo A, Abiola A, Dieng Y, Ndiaye D, Hallett R, Alifrangis M, Gaye O, 2012. Assessment of the molecular marker of *Plasmodium falciparum* chloroquine resistance (*Pfcr*) in Senegal after several years of chloroquine withdrawal. *Am J Trop Med Hyg* 87: 640–645.
151. Mang'era CM, Mbai FN, Omedo IA, Mireji PO, Omar SA, 2012. Changes in genotypes of *Plasmodium falciparum* human malaria parasite following withdrawal of chloroquine in Tiwi, Kenya. *Acta Trop* 123: 202–207.
152. Mita T, Kaneko A, Lum JK, Bwijo B, Takechi M, Zungu IL, Tsukahara T, Tanabe K, Kobayakawa T, Bjorkman A, 2003. Recovery of chloroquine sensitivity and low prevalence of the *Plasmodium falciparum* chloroquine resistance transporter

- gene mutation K76T following the discontinuance of chloroquine use in Malawi. *Am J Trop Med Hyg* 68: 413–415.
153. Kublin JG, Cortese JF, Njunju EM, Mukadam RA, Wirima JJ, Kazembe PN, Djimde AA, Kouriba B, Taylor TE, Plowe CV, 2003. Reemergence of chloroquine-sensitive *Plasmodium falciparum* malaria after cessation of chloroquine use in Malawi. *J Infect Dis* 187: 1870–1875.
 154. Thaithong S, Suebsaeng L, Rooney W, Beale GH, 1988. Evidence of increased chloroquine sensitivity in Thai isolates of *Plasmodium falciparum*. *Trans R Soc Trop Med Hyg* 82: 37–38.
 155. de Almeida A, Arez AP, Cravo PV, do Rosario VE, 2009. Analysis of genetic mutations associated with anti-malarial drug resistance in *Plasmodium falciparum* from the Democratic Republic of East Timor. *Malar J* 8: 59.
 156. Frank M, Lehnert N, Mayengue PI, Gabor J, Dal-Bianco M, Kombila DU, Ngoma GM, Supan C, Lell B, Ntoumi F, Grobusch MP, Dietz K, Kremsner PG, 2011. A thirteen-year analysis of *Plasmodium falciparum* populations reveals high conservation of the mutant *pfprt* haplotype despite the withdrawal of chloroquine from national treatment guidelines in Gabon. *Malar J* 10: 304.
 157. Kamugisha E, Bujila I, Lahdo M, Pello-Esso S, Minde M, Kongola G, Naiwumbwe H, Kiuwua S, Kaddumukasa M, Kironde F, Swedberg G, 2012. Large differences in prevalence of *Pfprt* and *Pfmdr1* mutations between Mwanza, Tanzania and Iganga, Uganda—a reflection of differences in policies regarding withdrawal of chloroquine? *Acta Trop* 121: 148–151.
 158. Mwai L, Ochong E, Abdirahman A, Kiara SM, Ward S, Kokwaro G, Sasi P, Marsh K, Borrmann S, Mackinnon M, Nzila A, 2009. Chloroquine resistance before and after its withdrawal in Kenya. *Malar J* 8: 106.
 159. McCollum AM, Mueller K, Villegas L, Udhayakumar V, Escalante AA, 2007. Common origin and fixation of *Plasmodium falciparum dhfr* and *dhps* mutations associated with sulfadoxine-pyrimethamine resistance in a low-transmission area in South America. *Antimicrob Agents Chemother* 51: 2085–2091.
 160. Babiker HA, 2009. Seasonal fluctuation of drug-resistant malaria parasites: a sign of fitness cost. *Trends Parasitol* 25: 351–352.
 161. Babiker HA, Hastings IM, Swedberg G, 2009. Impaired fitness of drug-resistant malaria parasites: evidence and implication on drug-deployment policies. *Expert Rev Anti Infect Ther* 7: 581–593.
 162. Ord R, Alexander N, Dunyo S, Hallett R, Jawara M, Targett G, Drakeley CJ, Sutherland CJ, 2007. Seasonal carriage of *pfprt* and *pfmdr1* alleles in Gambian *Plasmodium falciparum* imply reduced fitness of chloroquine-resistant parasites. *J Infect Dis* 196: 1613–1619.
 163. Ursing J, Kofoed PE, Rodrigues A, Rombo L, 2009. No seasonal accumulation of resistant *P. falciparum* when high-dose chloroquine is used. *PLoS One* 4: e6866.
 164. Asih PB, Rogers WO, Susanti AI, Rahmat A, Rozi IE, Kusumaningtyas MA, Krisin, Sekartuti, Dewi RM, Coutrier FN, Sutamihardja A, van der Ven AJ, Sauerwein RW, Syafruddin D, 2009. Seasonal distribution of anti-malarial drug resistance alleles on the island of Sumba, Indonesia. *Malar J* 8: 222.
 165. Mbacham WF, Evehe MS, Netongo PM, Ateh IA, Mimche PN, Ajua A, Nji AM, Irene D, Echouffo-Tcheugui JB, Tawe B, Hallett R, Roper C, Targett G, Greenwood B, 2010. Efficacy of amodiaquine, sulphadoxine-pyrimethamine and their combination for the treatment of uncomplicated *Plasmodium falciparum* malaria in children in Cameroon at the time of policy change to artemisinin-based combination therapy. *Malar J* 9: 34.
 166. Bhumiratana A, Intarapuk A, Sorosjinda-Nunthawarasilp P, Maneekan P, Koyadun S, 2013. Border malaria associated with multidrug resistance on Thailand-Myanmar and Thailand-Cambodia borders: transmission dynamic, vulnerability, and surveillance. *BioMed Res Int* 2013: 363417.
 167. Lukens AK, Ross LS, Heidebrecht R, Javier Gamo F, Lafuente-Monasterio MJ, Booker ML, Hartl DL, Wiegand RC, Wirth DF, 2014. Harnessing evolutionary fitness in *Plasmodium falciparum* for drug discovery and suppressing resistance. *Proc Natl Acad Sci USA* 111: 799–804.
 168. Lelievre J, Berry A, Benoit-Vical F, 2007. Artemisinin and chloroquine: do mode of action and mechanism of resistance involve the same protagonists? *Curr Opin Investig Drugs* 8: 117–124.
 169. Sisowath C, Petersen I, Veiga MI, Martensson A, Premji Z, Bjorkman A, Fidock DA, Gil JP, 2009. In vivo selection of *Plasmodium falciparum* parasites carrying the chloroquine-susceptible *pfprt* K76 allele after treatment with artemether-lumefantrine in Africa. *J Infect Dis* 199: 750–757.
 170. Froberg G, Jorhagen L, Morris U, Shakely D, Msellem MI, Gil JP, Bjorkman A, Martensson A, 2012. Decreased prevalence of *Plasmodium falciparum* resistance markers to amodiaquine despite its wide scale use as ACT partner drug in Zanzibar. *Malar J* 11: 321.
 171. Tukwasibwe S, Mugenyi L, Mbogo GW, Nankoberanyi S, Maiteki-Sebuguzi C, Joloba ML, Nsobyia SL, Staedke SG, Rosenthal PJ, 2014. Differential prevalence of transporter polymorphisms in symptomatic and asymptomatic falciparum malaria infections in Uganda. *J Infect Dis* 210: 154–157.
 172. Mharakurwa S, Sialumano M, Liu K, Scott A, Thuma P, 2013. Selection for chloroquine-sensitive *Plasmodium falciparum* by wild *Anopheles arabiensis* in southern Zambia. *Malar J* 12: 453.
 173. Mharakurwa S, Kumwenda T, Mkulama MA, Musapa M, Chishimba S, Shiff CJ, Sullivan DJ, Thuma PE, Liu K, Agre P, 2011. Malaria antifolate resistance with contrasting *Plasmodium falciparum* dihydrofolate reductase (DHFR) polymorphisms in humans and *Anopheles* mosquitoes. *Proc Natl Acad Sci USA* 108: 18796–18801.
 174. Mharakurwa S, 2004. *Plasmodium falciparum* transmission rate and selection for drug resistance: a vexed association or a key to successful control? *Int J Parasitol* 34: 1483–1487.
 175. Mharakurwa S, Mutambu SL, Mudyiradima R, Chimbadzwa T, Chandiwana SK, Day KP, 2004. Association of house spraying with suppressed levels of drug resistance in Zimbabwe. *Malar J* 3: 35.
 176. Al-Mekhlafi AM, Mahdy MA, Al-Mekhlafi HM, Azazy AA, Fong MY, 2011. High frequency of *Plasmodium falciparum* chloroquine resistance marker (*pfprt* T76 mutation) in Yemen: an urgent need to re-examine malaria drug policy. *Parasit Vectors* 4: 94.
 177. Alifrangis M, Lemnge MM, Ronn AM, Segeja MD, Magesa SM, Khalil IF, Bygbjerg IC, 2003. Increasing prevalence of wildtypes in the dihydrofolate reductase gene of *Plasmodium falciparum* in an area with high levels of sulfadoxine/pyrimethamine resistance after introduction of treated bed nets. *Am J Trop Med Hyg* 69: 238–243.
 178. Shah M, Kariuki S, Vanden Eng J, Blackstock AJ, Garner K, Gatei W, Gimnig JE, Lindblade K, Terlouw D, ter Kuile F, Hawley WA, Phillips-Howard P, Nahlen B, Walker E, Hamel MJ, Slutsker L, Shi YP, 2011. Effect of transmission reduction by insecticide-treated bednets (ITNs) on antimalarial drug resistance in western Kenya. *PLoS One* 6: e26746.