Artemisinin resistance: current status and scenarios for containment

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Abstract | Artemisinin combination therapies are the first-line treatments for uncomplicated *Plasmodium falciparum* malaria in most malaria-endemic countries. Recently, partial artemisinin-resistant *P. falciparum* malaria has emerged on the Cambodia–Thailand border. Exposure of the parasite population to artemisinin monotherapies in subtherapeutic doses for over 30 years, and the availability of substandard artemisinins, have probably been the main driving force in the selection of the resistant phenotype in the region. A multifaceted containment programme has recently been launched, including early diagnosis and appropriate treatment, decreasing drug pressure, optimising vector control, targeting the mobile population, strengthening management and surveillance systems, and operational research. Mathematical modelling can be a useful tool to evaluate possible strategies for containment.

Parenteral
Administered by injection.

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In nearly all countries in which malaria is endemic, artemisinin combination therapies (ACT) are now the recommended first-line therapy for uncomplicated *Plasmodium falciparum* malaria, a policy endorsed by the WHO[1]. This change in policy followed a period of increasing failure rates with chloroquine and later sulphadoxine–pyrimethamine treatment, which arose from the development of resistant *P. falciparum* strains. The spread and increased levels of resistance to the generally available and affordable drugs resulted in an increase in the number of deaths caused by malaria in children under 5 years of age in sub-Saharan Africa during a period when overall childhood mortality was decreasing[2–4]. This trend has now been reversed with the introduction of ACTs and other control measures, specifically the widespread use of insecticide-treated bed nets. A marked decrease in malaria burden has been observed in several Asian and African regions, where these effective control measures have been deployed actively[5–8]. In addition, parenteral artesunate, an artemisin derivative, became the treatment of choice for severe malaria in adults after it was shown to reduce mortality by 35% compared with quinine[9].

Artemisinins extracted from the ubiquitous annual wormwood *Artemisia annua* have been used in traditional Chinese medicine for more than 2,000 years for the treatment of febrile illnesses[10]. In the 1970s the chemical structure of a sesquiterpene peroxide with powerful antimalarial properties (artemisinin) was identified, and several more potent derivatives were synthesized, including artesunate, artemether and dihydroartemisinin[11] (FIG. 1). Artemisinin derivatives have an excellent safety profile in the treatment of malaria, a rapid onset of action and are active against the broadest range of stages in the life cycle of *Plasmodium* spp. compared with other antimalarials[11,12] (FIG. 2). Artemisinins also kill immature and developing gametocytes, the sexual stages that are essential for transmission[13,14], thereby reducing gametocyte carriage and infectivity.

In the blood, the dihydroartemisinin derivatives artesunate, artemether and artemotil are quickly and completely hydrolysed back to dihydroartemisinin, which has a short plasma half-life of ~ 1 hour[10]. A once or twice a day dosing regimen with artemisinin derivatives results in a reduction of four orders of magnitude of the asexual parasite biomass per 48-hour treatment cycle (FIG. 2). Despite this remarkable antimalarial activity, artemisin derivative monotherapy for 7 days covering 3 cycles of the asexual life cycle of the parasite is needed to completely eliminate a biomass of 10^{12} parasites, which corresponds to a parasitaemia of ~2% in an adult[11]. The short half-life of artemisinin derivatives minimizes the period available for the selection of resistant strains (known as the selective window)[15]. However, there is still the potential for the emergence of resistant strains when artemisinin derivatives are deployed

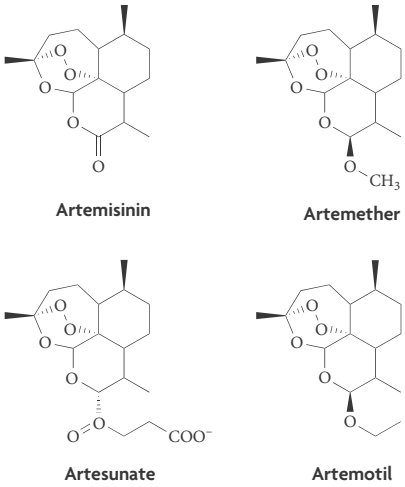


Figure 1 | **Chemical structure of artemisinins.** Artemisinin is the compound that is produced by the plant *Artemisia annua*. The derivatives arthemether and artesunate have better bioavailability than artemisinin and are used clinically in artemisinin combination therapy. Artemotil (also known as arteether) is infrequently used.

as monotherapies in areas of increasing drug pressure, so researchers suggested more than a decade ago that artemisinin derivatives should be used only in combination with partner drugs in ACTs[16]. The artemisinin component of ACTs rapidly kills the bulk of the organisms and a partner drug with a longer plasma half-life eliminates the remaining parasites. This not only optimizes the therapeutic benefit, but also mutually protects both components of the ACT and minimizes the risk of resistant parasites emerging and spreading[17]. In the absence of an efficient partner drug, repeated exposure to artemisinin monotherapies at subtherapeutic doses, especially in hyperparasitaemic patients and over prolonged periods of time, will provide a risk for the emergence of resistance[18].

After a lengthy delay before general acceptance, ACTs have now been implemented as first-line treatment in the national malaria control programmes of most malaria-endemic countries[19]. Deployment in the private sector, however, lags far behind. To ensure the use of the combination, rather than the individual components, fixed-dose ACTs have been developed, including artemether–lumefantrine, artesunate–mefloquine and artesunater–amodiaquine. Dihydroartemisinin–piperaquine and artesunate–pyronaridine are in advanced stages of clinical testing and drug registration. The short and mid-term pipeline for antimalarial drug development depends on artemisinin derivatives[20]; losing the artemisinin derivatives because of *P. falciparum* drug resistance would be a disaster for malaria control and treatment and would seriously threaten current malaria elimination efforts.

This Review presents the evidence that resistance to artemisinins has emerged in western Cambodia and discusses alternative therapies and treatment regimens that can decrease the spread and independent occurrence of drug resistance.

Recrudescence
Reoccurence of a disease after treatment. This can be caused by parasites that were not completely eliminated during the treatment.

Emergence of resistant *P. falciparum* strains

The first reports of higher recrudescence rates of *P. falciparum* malaria after treatment with ACTs emerged from observational data collected in Cambodia since 2004 (REFS 21,22). It was not clear initially whether these high failure rates resulted from resistance to artemisinins, their partner drugs or unusual host or pharmacokinetic factors[23,24]. A study carried out in 2006 and 2007 in Battambang province, Cambodia, showed that a minority of patients with uncomplicated *P. falciparum* malaria harboured parasites with decreased *in vitro* sensitivity to artesunate and showed delayed parasite clearance times in the presence of apparently adequate plasma drug concentrations after treatment with artesunate in a dose of 4 mg per kg per day for 7 days[25].

Conclusive evidence came from a recent study comparing the therapeutic responses to artesunate in patients with uncomplicated *P. falciparum* malaria in Pailin, western Cambodia, and Wang Pha, western Thailand, where artemisinin derivatives remain effective[26] (FIG. 3). Clearance rates were much slower in western Cambodia and showed little heterogeneity (FIG. 4). Specifically, after artesunate monotherapy in a dose of 2 mg per kg per day for 7 days or artenusate in a dose of 4 mg per kg per day for 3 days followed by mefloquine in a dose of 25 mg per kg per day, the median parasite clearance time was 84 hours (interquartile range (IQR) = 60 to 96) in Pailin compared with 48 hours (IQR = 36 to 66) in Wang Pha (p=0.001), with similar drug concentration profiles in both sites. The difference in clearance rates was not explained by genetic polymorphisms in the *P. falciparum* genes *pfcrt* (chloroquine resistance transporter gene), *pfmdr1* (multidrug resistance gene 1) or *pfserca* (a sacroplasmic reticulum Ca^{2+} ATPase) — which had been suggested previously as the targets of artemisinins — or amplification in *pfmdr1*. Heritability studies suggest that the observed artemisinin resistance phenotype of the parasites has a genetic basis and thus is expected to spread within parasite populations that live where artemisinins are deployed unless associated fitness costs of the putative resistance mutation or mutations outweigh selective benefits[27]. The study shows that in patients treated with artesunate, genetically identical parasite strains (defined by microsatellite typing) strongly cluster in patients with slow versus fast parasite clearance rates. To date, the molecular basis for the resistance mechanism remains unknown, although intensive molecular and phenotypical characterization is under way.

Development of resistance

Several factors may have contributed to the emergence of reduced artemisinin sensitivity in Cambodia. Cambodia was one of the first countries to adopt ACTs as first-line treatment in 2001, but unregulated artemisinin or artesunate monotherapy has been available since the mid-1970s. A recent survey showed that 78% of patients obtain their antimalarial treatment through the private sector, mostly as artesunate monotherapy[28]. The problem is compounded by the unavailability of the fixed dose combination of the current first-line ACT

artesunate–mefloquine. Although it has been developed as a fixed dose combination, it is currently available only as separate tablets, facilitating the continued use of artemisin or artesunate monotherapy. Counterfeited or substandard tablets that contain less active ingredients than stated are additional sources of subtherapeutic dosing of artemisinins, which may also have contributed to the selection of resistant parasite strains[29]. Moreover, it is possible that the different pharmacokinetic properties of artemisinins in subgroups of the population, such as pregnant women and children, have resulted in underdosing.

Why has this problem emerged in western Cambodia and not in other parts of Southeast Asia with similar conditions? We think two factors have been vital. First, there has been uniquely massive drug pressure. Second, the low malaria transmission in the area was probably essential to allow resistant parasite populations to establish themselves[31,32]. In addition, it is possible that parasite factors, such as a unique *P. falciparum* phenotype[30], or host factors have played a part[18,31,32].

Western Cambodia has previously been a focal point for the emergence of chloroquine resistance[33] and for sulphadoxin–pyrimethamine resistance[34] (BOX 1). The relative affluence generated by the mining of precious stones in the area has attracted a highly mobile, susceptible population into an area where malaria is transmitted and has supported informal sales of antimalarials. Migrants working in the area are an added concern as they could carry and spread artemisinin-resistant strains to other countries. However, 5 years after the first indications of reduced susceptibility to artemisinins, the clearly resistant *P. falciparum* phenotype is still confined to the Cambodia–Thailand border, although parasite clearance times after treatment with artemisinin derivatives have also, but to a much lesser extent, increased on the border of Thailand and Myanmar (formerly known as Burma)[35]. It should also be noted that ACTs are still effective in western Cambodia, with cure rates usually exceeding 90%, as resistance is not complete and the parasites are still killed by artemisinins, albeit at much lower rates. It will be of utmost importance to continue monitoring the spread of the artemisinin-resistant phenotype through the region. Because a sensitive *in vitro* test and a molecular marker for artemisinin resistance is not currently available, clinical monitoring of the affected population is necessary.

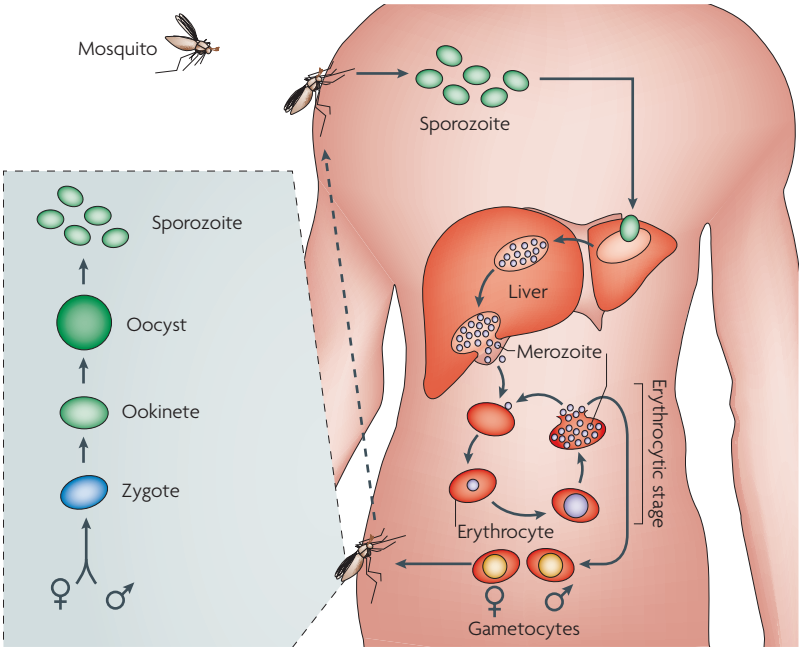


Figure 2 | **The life cycle of *Plasmodium falciparum*.** The infection in humans is initiated by the bite of a mosquito, which releases sporozoites in the bloodstream. The sporozoites infect hepatocytes and undergo many rounds of replication, ultimately releasing merozoites. These invade erthrocytes, leading to the symptoms of malaria. Infection of erythrocytes results in the formation of additional merozoites, allowing the infection to expand. A small percentage of merozoites differentiates to form male or female gametocytes. After these are taken up by a mosquito during a bloodmeal, they convert to male and female gametes and fuse to form a zygote. Subsequently development through the ookinete and oocyst stages leads to the formation of sporozoites, which migrate to the salivary glands of the mosquito, from which they can be injected into the host. Artemesinin targets the parasites in the erythocytic stages, the merozoites and the gametocytes, preventing both their growth and spread.

Treatment during artemisinin failure

There is currently no group of drugs that can replace artemisinins in the way that sulphadoxine–pyrimethamine replaced chloroquine and ACTs replaced sulphadoxine–pyrimethamine. Semisynthetic artemisinins and synthetic endoperoxides have the same advantages as the artemisinin derivatives used in ACTs (that is, rapid parasite killing and broad stage specificity) and will undergo clinical testing in the near future, but it is uncertain whether these new drugs will be more effective against artesunate-resistant parasites than the currently available ones.

None of the currently licensed antimalarial drugs has a similar safety and efficacy profile to the artemisinin derivatives. The non-artemisinin-based compounds that are in the early stages of development are likely to take at least a decade until they become available for clinical use[20]. Quinine remains useful for cerebral malaria and other forms of severe malaria, but a 7-day course is needed for the complete treatment of uncomplicated malaria. Furthermore, patients are unlikely to adhere to a full course of quinine because of its frequent adverse events, three times per day dosing and an unpalatable taste surprisingly. Not surprisingly, recrudescence is observed frequently[36].

One of the few remaining effective drugs without an artemisinin derivative component is the combination of atovaquone–proguanil. The use of atovaquone–proguanil has been limited to prevention and treatment in travellers, not because of a lack of efficacy or safety concerns, but because of its prohibitively high price. The lack of availability has so far minimized the drug pressure and prevented the appearance of resistance, but a single point mutation in codon 268 in the cytochrome *b* gene of *P. falciparum* confers a high level of atovaquone resistance. This rapid emergence of resistance to atovaquone despite optimal dosing suggests that the drug would have a short lifespan if it were widely used for malaria treatment[37].

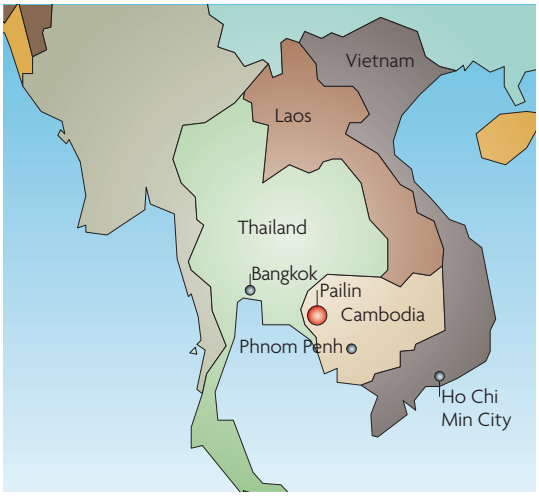


Figure 3 | **The study site in Pailin, western Cambodia.** Decreased artemisinin sensitivity was first detected in Pailin, western Cambodia, near the border with Thailand.

Treatment of artemisinin-resistant malaria will rely on optimizing the combinations of existing drugs. Four antimalarials (lumefantrine, mefloquine, amodiaquine and sulphadoxine–pyrimethamine) are widely used in the combination with artemisinin derivatives. However, amodiaquine[38] and sulphadoxine–pyrimethamine[39] resistance is already widespread, and although lumefantrine and mefloquine can still be used everywhere[40], resistance could be readily selected, particular if monotherapies were deployed[41].

Drugs that have been more recently included in ACTs include piperaquine and pyronaridine. Piperaquine was used extensively as a treatment in China[42], which led to the rapid selection of resistance locally; however, it has retained efficacy elsewhere and has proved to be a valuable partner drug in combination with dihydroartemisinin[43]. The dihydroartemisinin–piperaquine combination still awaits pre-qualification by the WHO, but it is already widely used in Southeast Asia. Indeed, the Ministry of Health in Cambodia has recently changed its first-line treatment of uncomplicated *P. falciparum* malaria to dihydroartemisinin–piperaquine in the affected regions in western Cambodia. Pyronaridine is a newer drug that is likely to be licensed next year as a co-formulation with artenusate[44,45]. Pyronaridine on its own is an effective and apparently safe antimalarial[46]. As there has been minimal use of the pyronaridine components as monotherapy, the combination with artenusate holds considerable promise.

Another possibility is the simultaneous administration of three or more drugs, which is recommended and widely accepted for other diseases, including tuberculosis, multibacillary leprosy, *Helicobacter pylori* gastric ulcers and AIDS[47,48]. Triple therapy, including an artemisinin derivative and two partner drugs with matching plasma terminal half-lives, has yet to be fully explored for the treatment of uncomplicated *P. falciparum* malaria. A practical difficulty in the development of triple therapies is the increased potential for drug interactions and a longer time frame for approval from regulatory institutions. Adding a gametocytocidal drug to existing ACTs would be ideal to block or at least minimize the transmission and hence the spread of artemisinin-resistant malaria.

The only current gametocytocidal drugs are 8-aminoquinolones, such as primaquine. Primaquine is effective against mature-stage gametocytes, but has low activity against the erythrocytic stages in the lifecycle of *P. falciparum*. When given as a 7-day course as an adjunct to an ACT, primaquine accelerates gametocyte clearance[14]. Whether a single dose of primaquine given on the last day of a course of an ACT has a similar effect has not been well studied. Nevertheless, single dose primaquine has been widely recommended by malaria control programmes for many years (although often not actually used). Modelling suggests that primaquine, which has a short half-life of approximately 8 hours, should ideally be given as a follow-up treatment to ACT 8 days after the start of ACT[49]. This is because gametocytaemia tends to peak about a week after the initial acute attack. The same model also suggests that the intervention should be deployed only on top of an effective antimalarial drug combination that kills the erythrocytic stages of the parasite.

Another problem for deployment is that the safety profile of primaquine, which has not been well studied, has bedeviled the treatment of hypnozoites[50]. Primaquine and 8-aminiquinolones have oxidative properties, and these cause intravascular haemolysis in individuals with glucose-6-phosphate dehydrogenase (G6PD) deficiency[2]. G6PD deficiency is an X-linked, hereditary genetic defect that results from different mutations in *G6PD* and has many biochemical and clinical phenotypes[51]. At least 140 mutations have been described, which differ extensively with regard to the severity of the corresponding reduction in G6PD activity. The risk–benefit assessment for the use of primaquine as a transmission blocking agent in settings in which parasite transmission is low and elimination might be possible[52] is currently being assessed. The benefit to the community of reduced malaria transmission should be balanced with the risk to the individual patient who derives no personal direct benefit from blocking transmissibility. These safety concerns currently present a barrier for the acceptance of 8-aminoquinolones in triple therapy combinations.

Delaying the spread of artemisinin resistance

Several public health strategies to interrupt the spread and prevent further emergence of artemisinin resistance are under consideration, and recently a programme was launched for the containment of partial artemisinin resistance in western Cambodia and eastern Thailand[53]. The proposed programme involves a multifaceted approach, including early diagnosis and appropriate treatment of malaria, decreasing the drug pressure, optimizing insect vector control, targeting the mobile population, strengthening disease management and surveillance systems, and operations research. The technical, operational and financial feasibility of interventions depends on the epidemiology of malaria, quality of

Hypnozoite
A dormant form of the liver stage parasites found in several *Plasmodium* spp., including the human parasites *Plasmodium vivax* and *Plasmodium ovale*.

existing health infrastructures, skilled manpower, funding, health-seeking behaviour, free-market forces, and regulatory and ultimately political forces. Each of these parameters varies between regions. For example, interventions that are suitable for western Cambodia could be inappropriate for parts of Thailand. As the empirical testing of some strategies would be prohibitively slow and expensive, choosing the optimal approach requires reliance on observational data, expert opinion and predictive modelling.

Increasing coverage with effective antimalarial treatment. To ensure cure and reduce further transmission of resistant infections, it is essential that symptomatic patients are diagnosed promptly and treated effectively[16]. Therefore, patients need to have easy access to affordable, high-quality and effective treatment. Cambodia has applied to pilot the Affordable Medicine Facility — malaria (AMFm; see AMFm website), an innovative financing mechanism that aims to lower the price of good-quality ACTs sold in the public and private sector and to not-for-profit buyers. This effort will be funded by the Global Fund for HIV, Tuberculosis and Malaria, which will pay a large proportion of a negotiated low price directly to manufacturers on behalf of the buyers. This means that buyers will only pay approximately US$0.05 for each course of an ACT. However, it will only be appropriate to subsidize a fixed-dose ACT and not the current combination of two individual pills of artesunate and mefloquine.

Efforts are being made in western Cambodia to strengthen the capacity for malaria diagnosis and treatment in public primary healthcare facilities to ensure early diagnosis and adequate treatment. Lay village malaria workers are also being trained to provide free malaria diagnosis using rapid diagnostic tests and treatment with a good-quality ACT. This will also help to encourage patients to take the full course of the treatment, thereby avoiding the selection for antimalarial drug resistance.

Reduction of drug pressure. A strategic priority is to reduce the drug pressure exerted on parasites. The limited and controlled use of atovaquone–proguanil is being explored, but the artemisinin-based drugs will continue to be the basis of malaria treatment. This is particularly problematic in Cambodia, where the use of artemisinin monotherapies, substandard drugs and subtherapeutic doses of treatment courses in the private sector is common. The sale of oral artemisinin derivative monotherapies in the private sector has recently been banned in Cambodia, and there are ongoing efforts to strengthen the capacity for drug quality monitoring, regulation of the private sector and law enforcement. However, this remains a challenging area, and until a cheap, effective and high quality fixed-dose ACT becomes available in sufficient quantities, the problem is likely to continue. The AMFm initiative described above could prove to be an effective mechanism to push substandard and monotherapy antimalarials out of the market. Deployment of multiple first-line therapies (MFTs) is another strategy to reduce drug pressure on the parasite pool (discussed below).

Mass drug administrations. One approach to eliminate resistant malaria from a limited area is to administer a complete course of effective therapy to the whole population, irrespective of disease status. Several mass drug administrations (MDAs) to control malaria have been reported over the past 75 years[42]. However, the logistic challenges are formidable. A fraction of the population invariably refuses to participate, and adherence to multidose regimens is likely to be incomplete. To interrupt transmission a gametocytocidal drug has to be added, and to assure high coverage the therapy has to be free of side effects. Perhaps most importantly, MDAs result in a massive increase in drug pressure, which, as discussed above, should be minimized. Past MDAs have succeeded in reducing parasite prevalence and the incidence of clinical malaria but have failed to interrupt malaria transmission. Models predict that one round of MDA will not have an impact because the acceptable drug regimens fail to remove all gametocytes and cannot therefore break the transmission cycle[54].

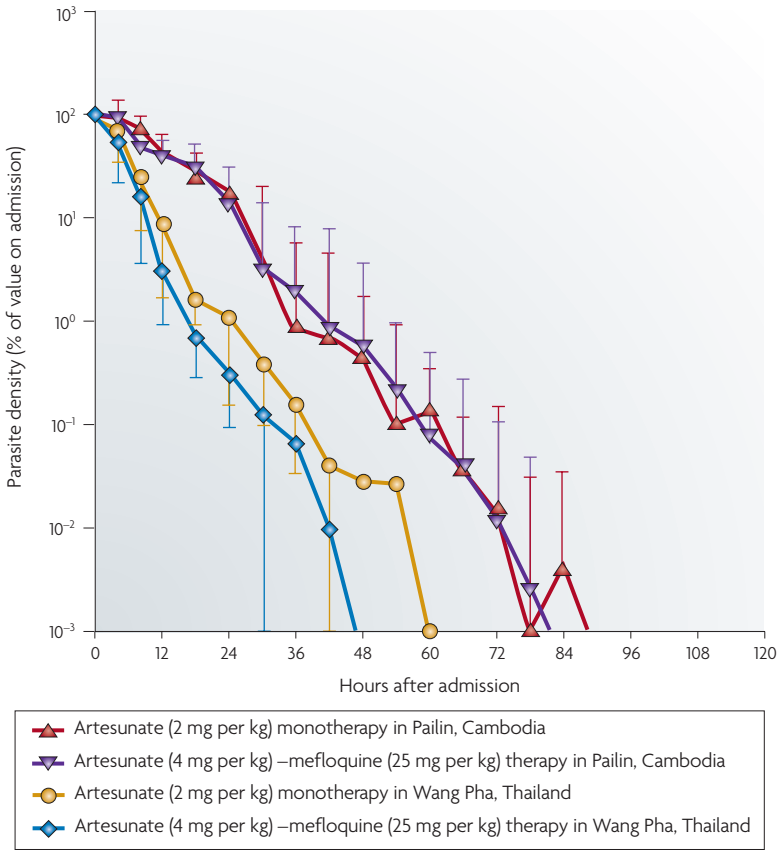
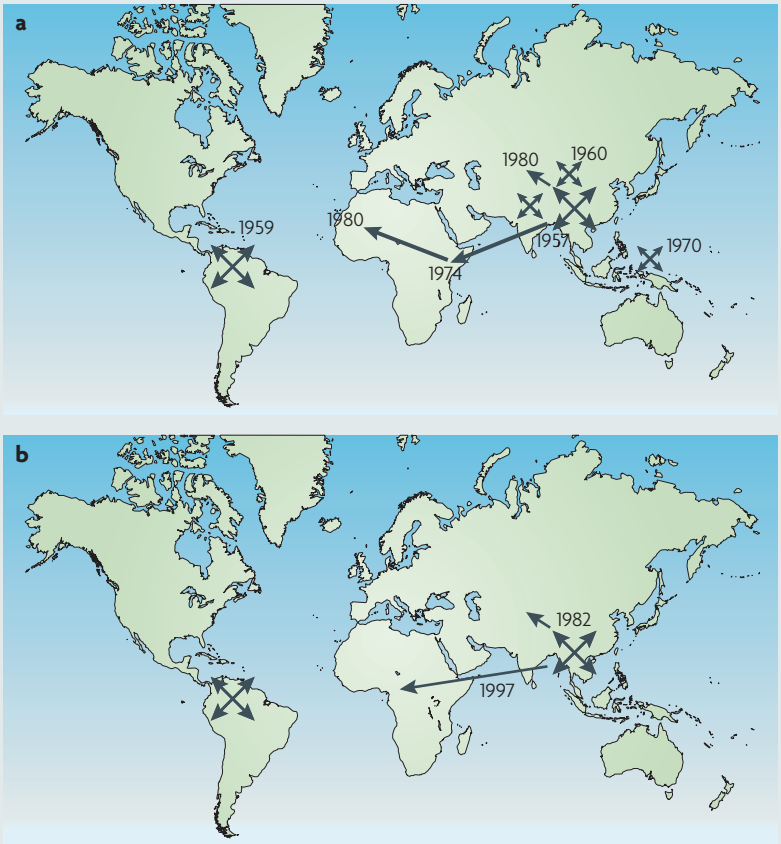


Figure 4 | **Parasite clearance rates.** Parasite clearance rates in patients with uncomplicated *P. falciparum* malaria in Pailin (western Cambodia) and Wang Pha (western Thailand). These study results clearly show that the parasites in patients from the Pailin region in Cambodia require longer treatment with either artesunate (shown in red) or artesunate–mefloquine (shown in purple) therapy than the parasites from the Wang Pha region in Thailand (shown in yellow and blue) to be cleared. Figure is reproduced, with permission, from REF. 26 © (2009) Massachusetts Medical Society.

Box 1 | **Chloroquine resistance**



Chloroquine was discovered by Andersag and co-workers in 1934 and patented 1939 by I.G Farbenindustrie AG[60]. Following several pitfalls and errors the drug became available for the treatment of malaria only from 1946 onwards[61]. Chloroquine had an enormous impact on the treatment of malaria and has probably saved hundreds of millions of lives[61]. It was hoped that chloroquine would have a pivotal role in the WHO malaria eradication programme, which begun in 1955 (REF. 62). However, chloroquine resistance was first reported in 1957 — the first documented cases of chloroquine resistance were observed in the Cambodia–Thailand border. Independently, chloroquine resistance emerged in South America in 1959, and was reported in Africa 17 years after the first cases in Asia. However, once choloroquine-resistant *Plasmodium falciparum* strains had gained a foothold in Africa, they dispersed rapidly from country to country[63]. By the early 1990s chloroquine had become useless for the treatment of *P. falciparum* malaria in east Africa and gradually became useless in west Africa as well over the rest of the decade[61]. The figure part **a** shows the global spread of chloroquine resistance; arrows represent the routes of spread and dates indicate the year of arrival of clinically significant antimalarial drug resistance in that region.

Chloroquine resistance is caused by multiple mutations in *pfcrt* (chloroquine resistance transporter gene), which encodes a transmembrane protein that is present in the digestive vacuole of *Plasmodium* parasites. The Lys76Thr mutation in this gene confers chloroquine resistance, but only in a genetic a background with several additional mutations in the same gene[64,65]. Molecular epidemiological analysis of *pfcrt* mutations and surrounding microsatellites have shown that the lineages from South American and Southeast Asia with the mutation in *pfcrt* are distinct and that the *pfcrt* that spread through Africa originated from Southeast Asia[66].

A recovery in chloroquine sensitivity following the halt of chloroquine use over a period of 10 years has been observed in Malawi, and this coincided with a decrease in the prevalence of the Lys76Thr mutation, suggesting a fitness disadvantage owing to the presence of the mutation in the absence of drug pressure[67,68]. As Malawi is surrounded by countries with extensive chloroquine resistance, reintroduction of chloroquine in Malawi is currently no option, as resistant parasites would probably return rapidly, imported in people from neighbouring areas.

When the utility of chloroquine decreased, sulphadoxine–pyrimethamine became an important treatment. However, high level resistance to pyrimethamine spread rapidly (see the figure, part **b**). Interestingly, resistance to the drug emerged in the same areas as chloroquine resistance (the Amazon basin and Southeast Asia). Artemisinin resistance has now emerged in one of these regions, Southeast Asia.

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In settings such as the Cambodia–Thailand border, where parasite prevalence is low, most of the population receiving MDAs will not harbour malaria parasites. In this case it has been suggested that the population should be screened, and parasitaemic individuals should be targeted for treatment. Mass screening and treatment (MSAT) has not been empirically tested, and there are concerns that it will not be possible to detect low-density parasitaemias and achieve the coverage rates that are needed to affect transmission. However, the effect of MSAT on the spread of resistant malaria has been modelled. In combination with the successful replacement of artemisinin monotherapies with ACTs for presumptive treatment, the model predicts that the absolute number of infections could be reduced by MSAT but the proportion of resistant infections may increase[54]. Furthermore, MSAT would have to be repeated regularly for almost a decade, as terminating

MSAT campaigns early would result in resurgence of infections with an increased proportion of artemisinin resistance.

Modelling suggests that to eliminate artemisinin resistance using ACT, all *P. falciparum* malaria has to be eliminated, as the last surviving parasite strains will be the most resistant ones[54]. It is therefore important to decrease the parasite burden in the community as low as possible and then treat the remaining artemisinin resistant strains with different drugs. However, there are only limited options to use non-ACT antimalarials for MDA or MSAT. Resistance levels to chloroquine and sulphadoxin–pyrimethamine are high and fixed in Southeast Asia. Atovaquone–proguanil could be used instead but, as discussed above, the parasites can become resistant through a single point mutation in the cytochrome *b* gene, which confers an up to 10,000-fold reduction in sensitivity to atovaquone[55].

Surveillance, active case investigation and focal control. Instead of broad regional mass population approaches, which can be used in the end phase of an malaria elimination programme, interventions that target foci of infection are likely to be a more effective and sustainable method to reduce the parasite burden in the region. For active case investigation and focal control, a well-functioning surveillance system is essential as are dedicated and motivated teams to follow up patients and carry out local intensive malaria control measures such as screening and treating nearby households and controlling the insect vector population. Although logistically still difficult, the routine screening and treatment of populations at risk of spreading resistant malaria, such as miners, loggers and military families, is likely to be effective.

Multiple first-line therapies. It has been proposed that varying first-line antimalarial therapies by area or age group could be an effective strategy to treat malaria[56]. Temporal cycling of insecticides is currently used to minimize the emergence of resistance[57] and could also be applied to first-line therapies. In this case, a population that harbours parasites that have developed resistance to the first drug in the cycling sequence would be treated with another drug after a set amount of time, decreasing the level of the resistant parasites. An evolutionary–epidemiological model based on clinical data from eight endemic regions in sub-Saharan Africa suggests that population-wide use of MFTs using three different drugs simultaneously in a population can reduce the emergence and spread of resistance in almost all transmission settings compared with a single first-line therapy or temporal cycling policies[58]. A population level combination of partner drugs is likely to be less effective than the application of triple therapies but it can be applied immediately and will provide some protection of the partner drugs while triple therapies are being developed and licensed. The logistical difficulties in adding and changing antimalarial policy should not be underestimated[59]. In reality MFT is already the prevailing practice in many countries such as Cambodia, where the private sector has an important role in providing malaria treatment and where there is a range of antimalarial products. Thus, in practice it might not be necessary to promote MFT actively but instead to encourage the availability of multiple effective drugs in the private sector.

Other malaria control measures. Other malaria control measures at the population level include vector control and, in the future, vaccines, although the most advanced malaria vaccine candidate is at least 5 years away from being made available to the public. In the meantime a goal of 100% coverage with long-lasting insecticide-treated nets (LLINs) is being vigorously pursued on the Cambodia–Thailand border and includes the use of insecticide-treated hammock nets for forest workers. Unfortunately, the main malaria vector species in this area, *Anopheles minimus*, *Anopheles maculatus* and *Anopheles dirus*, start feeding early in the evening, making the use of bed nets less effective than in sub-Saharan Africa. However, modelling work indicates that high coverage with LLINs as part of an elimination programme in the region could result in an estimated reduction in transmission of 30%, which could halve the time it takes to eliminate malaria completely[54]. Similarly, although indoor residual spraying has been shown to be effective in southern Africa[5], it is likely to be much less effective in the Cambodia–Thailand border because the vectors are exophilic (that is, they have a preference for resting outdoors rather than indoors). However, they might have an effect when being used in selected regions. There is also a need to explore alternative methods of vector control, including protection for individuals through the use of insect repellents and insect repellent clothing.

Conclusions

Partial artemisinin resistance has emerged in western Cambodia that is characterized by much slower parasite clearance rates after artemisinin treatment. The resistant strains have the potential to spread to different parts of the region and to subsequently become a global threat for malaria control and treatment. There are currently no alternative drugs to replace artemisinin derivatives. Atovaquone–proguanil is too expensive to deploy on a large scale and is prone to resistance development. Treatment of resistant *P. falciparum* strains therefore has to rely on the use of ACTs containing a potent partner drug, such as piperaquine or pyronaridine, which have not yet been compromised by resistance. Affordable high-quality, fixed combination ACTs should be made universally available to completely displace the artemisinin monotherapies and substandard drugs. Triple therapy has not yet been formally evaluated but should probably be developed and assessed before its use becomes necessary because of the development of resistance to artemisinins and multiple partner drugs. The addition of an 8-aminoquinolone as a gametocytocidal drug would be important to reduce transmission, but its safety needs to be further assessed in regions with a high prevalence of G6PD deficiency, such as western Cambodia.

Containing the problem in western Cambodia requires a multifaceted approach. Mathematical models are useful tools to evaluate possible strategies, and these suggest that MFTs hold greater promise to contain the spread of artemisinin resistance than a single first-line treatment. Ultimately, regional malaria elimination seems the only way to solve the problem, as the proportion of resistant parasites will increase with continued artemisinin exposure in a shrinking parasite pool. The WHO has launched a containment programme for western Cambodia and Thailand: this includes strengthening of early diagnosis and treatment programmes, banning artemisinin monotherapies in the private sector, increasing impregnated bed net coverage and documenting the migration of workers. Achieving effective control of malaria will crucially depend on the successful implementation of these strategies. The world cannot afford to lose artemisinin and artemisinin derivatives in the fight against malaria.

1. World Health Organisation. WHO guidelines for the treatment of malaria. (WHO, Geneva, 2006).
2. Baird, J. K. Effectiveness of antimalarial drugs. *N. Engl. J. Med.* **352**, 1565–1577 (2005).
3. Korenromp, E. L., Williams, B. G., Gouws, E., Dye, C. & Snow, R. W. Measurement of trends in childhood malaria mortality in Africa: an assessment of progress toward targets based on verbal autopsy. *Lancet Infect. Dis.* **3**, 349–358 (2003).
One of the few studies directly linking antimalarial drug resistance to an increase in mortality of African children that is due to malaria.
4. Trape, J. F. *et al.* Impact of chloroquine resistance on malaria mortality. *C. R. Acad. Sci. III* **321**, 689–697 (1998).
5. Barnes, K. I. *et al.* Effect of artemether-lumefantrine policy and improved vector control on malaria burden in KwaZulu-Natal, South Africa. *PLoS Med.* **2**, e330 (2005).
The first of several studies from a wide range of geographical areas showing the contribution ACTs to a reduction in malaria burden,
6. Bhattarai, A. *et al.* Impact of artemisinin-based combination therapy and insecticide-treated nets on malaria burden in Zanzibar. *PLoS Med.* **4**, e309 (2007).
7. Carrara, V. I. *et al.* Deployment of early diagnosis and mefloquine-artesunate treatment of falciparum malaria in Thailand: the Tak Malaria Initiative. *PLoS Med.* **3**, e183 (2006).
8. O'Meara, W. P. *et al.* Effect of a fall in malaria transmission on morbidity and mortality in Kilifi, Kenya. *Lancet* **372**, 1555–1562 (2008).
9. Dondorp, A., Nosten, F., Stepniewska, K., Day, N. & White, N. Artesunate versus quinine for treatment of severe falciparum malaria: a randomised trial. *Lancet* **366**, 717–725 (2005).
Large trial from Southeast Asia showing a 35% reduction in mortality in patients with severe malaria that were treated with artesunate compared with those treated with quinine.
10. Meshnick, S. R., Taylor, T. E. & Kamchonwongpaisan, S. Artemisinin and the antimalarial endoperoxides: from herbal remedy to targeted chemotherapy. *Microbiol. Rev.* **60**, 301–315 (1996).
11. White, N. J. Qinghaosu (artemisinin): the price of success. *Science* **320**, 330–334 (2008).
Excellent overview on the artemisinin derivatives as the most potent antimalarial drugs to date.
12. Adjuik, M. *et al.* Artesunate combinations for treatment of malaria: meta-analysis. *Lancet* **363**, 9–17 (2004).
Meta-analysis showing the superiority of ACTs for the treatment of uncomplicated *P. falciparum* malaria.
13. Okell, L. C., Drakeley, C. J., Ghani, A. C., Bousema, T. & Sutherland, C. J. Reduction of transmission from malaria patients by artemisinin combination therapies: a pooled analysis of six randomized trials. *Malar. J.* **7**, 125 (2008).
14. Pukrittayakamee, S. *et al.* Activities of artesunate and primaquine against asexual- and sexual-stage parasites in falciparum malaria. *Antimicrob. Agents Chemother.* **48**, 1329–1334 (2004).
15. Stepniewska, K. & White, N. J. Pharmacokinetic determinants of the window of selection for antimalarial drug resistance. *Antimicrob. Agents Chemother.* **52**, 1589–1596 (2008).
16. White, N. J. & Olliaro, P. L. Strategies for the prevention of antimalarial drug resistance: rationale for combination chemotherapy for malaria. *Parasitol. Today* **12**, 399–401 (1996).
17. White, N. J. Antimalarial drug resistance. *J. Clin. Invest.* **113**, 1084–1092 (2004).
Review on the theoretical background and mechanisms underlying emergence and spread of antimalarial drug resistance.
18. White, N. J. *et al.* Hyperparasitaemia and low dosing are an important source of anti-malarial drug resistance. *Malar. J.* **8**, 253 (2009).
19. World Health Organisation. World Malaria Report 2008. (WHO, Geneva, 2008).
20. Olliaro, P. & Wells, T. N. The global portfolio of new antimalarial medicines under development. *Clin. Pharmacol. Ther.* **85**, 2584–595 (2009).
Overview of the current portfolio of new antimalarial drugs, almost exclusively relying on ACTs.
21. Resistance to artemisinin derivatives along the Thai-Cambodian border. *Wkly Epidemiol. Rec.* **82**, 360 (2007).
22. Denis, M. B. *et al.* Surveillance of the efficacy of artesunate and mefloquine combination for the treatment of uncomplicated falciparum malaria in Cambodia. *Trop. Med. Int. Health* **11**, 1360–1366 (2006).
23. Alker, A. P. *et al. Pfmdr1* and *in vivo* resistance to artesunate-mefloquine in falciparum malaria on the Cambodian-Thai border. *Am. J. Trop. Med. Hyg.* **76**, 641–647 (2007).
24. Wongsrichanalai, C. & Meshnick, S. R. Declining artesunate-mefloquine efficacy against falciparum malaria on the Cambodia-Thailand border. *Emerg. Infect. Dis.* **14**, 716–719 (2008).
25. Noedl, H., Socheat, D. & Satimai, W. Artemisinin-resistant malaria in Asia. *N. Engl. J. Med.* **361**, 540–541 (2009).
One of the first detailed reports on emerging artemisinin resistance in western Cambodia.
26. Dondorp, A. M. *et al.* Artemisinin resistance in *Plasmodium falciparum* malaria. *N. Engl. J. Med.* **361**, 455–467 (2009).
Hallmark study showing a clear reduction in *in vivo P. falciparum* susceptibility to artesunate on the Cambodia–Thailand border.
27. Anderson, T. *et al.* High heritability of malaria parasite clearance rates indicates a genetic basis for artemisinin resistance in Western Cambodia. *J. Infect. Dis.* (in the press).
28. Yeung, S., Van Damme, W., Socheat, D., White, N. J. & Mills, A. Access to artemisinin combination therapy for malaria in remote areas of Cambodia. *Malar. J.* **7**, 96 (2008).
Study showing that treatment of uncomplicated *P. falciparum* malaria in remote areas of Cambodia is mainly through the less well-controlled private sector, in which artemisinin monotherapies are widely available.
29. Newton, P. N., Dondorp, A., Green, M., Mayxay, M. & White, N. J. Counterfeit artesunate antimalarials in southeast Asia. *Lancet* **362**, 169 (2003).
Article highlighting the terrible problem of the wide availability of counterfeited artemisinins in Southeast Asia.
30. Rathod, P. K., McErlean, T. & Lee, P. C. Variations in frequencies of drug resistance in *Plasmodium falciparum*. *Proc. Natl Acad. Sci. USA* **94**, 9389–9393 (1997).
31. Pongtavornpinyo, W. *et al.* Spread of anti-malarial drug resistance: mathematical model with implications for ACT drug policies. *Malar. J.* **7**, 229 (2008).
Modelling paper investigating the minimal public health conditions, such as ACT coverage rates, that are necessary to delay the spread of antimalarial drug resistance.
32. Maude, R. J. *et al.* The role of mathematical modelling in malaria elimination and eradication. *Trans. R. Soc. Trop. Med. Hyg.* **103**, 643–644 (2009).
33. Verdrager, J. Epidemiology of the emergence and spread of drug-resistant falciparum malaria in South-East Asia and Australasia. *J. Trop. Med. Hyg.* **89**, 277–289 (1986).
34. Verdrager, J. Localized permanent epidemics: the genesis of chloroquine resistance in *Plasmodium falciparum*. *Southeast Asian J. Trop.Med. Public Health* **26**, 23–28 (1995).
35. Carrara, V. I. *et al.* Changes in the treatment responses to artesunate-mefloquine on the northwestern border of Thailand during 13 years of continuous deployment. *PLoS ONE* **4**, e4551 (2009).
First study reporting a slight decrease in treatment responses to an ACT for uncomplicated *P. falciparum* malaria in an area outside the Cambodia–Thailand border region.
36. Achan, J. *et al.* Effectiveness of quinine versus artemether-lumefantrine for treating uncomplicated falciparum malaria in Ugandan children: randomised trial. *BMJ* **339**, b2763 (2009).
37. Gebru, T., Hailu, A., Kremsner, P. G., Kun, J. F. & Grobusch, M. P. Molecular surveillance of mutations in the cytochrome *b* gene of *Plasmodium falciparum* in Gabon and Ethiopia. *Malar. J.* **5**, 112 (2006).
38. Olliaro, P. & Mussano, P. Amodiaquine for treating malaria. *Cochrane Database Syst. Rev.* **2003**, CD000016 (2003).
39. Gesase, S. *et al.* High resistance of *Plasmodium falciparum* to sulphadoxine/pyrimethamine in northern Tanzania and the emergence of dhps resistance mutation at codon 581. *PLoS ONE* **4**, e4569 (2009).
40. Pilz, J. B. *et al. In vitro* sensitivity of *Plasmodium falciparum* to lumefantrine in north-western Thailand. *Wien. Klin. Wochenschr.* **116** (Suppl. 4), 41–46 (2004).
41. Wongsrichanalai, C., Pickard, A. L., Wernsdorfer, W. H. & Meshnick, S. R. Epidemiology of drug-resistant malaria. *Lancet Infect. Dis.* **2**, 209–218 (2002).
42. von Seidlein, L. & Greenwood, B. M. Mass administrations of antimalarial drugs. *Trends Parasitol.* **19**, 452–460 (2003).
43. Tran, T. H. *et al.* Dihydroartemisinin-piperaquine against multidrug-resistant *Plasmodium falciparum* malaria in Vietnam: randomised clinical trial. *Lancet* **363**, 18–22 (2004).
44. Ramharter, M. *et al.* Fixed-dose pyronaridine-artesunate combination for treatment of uncomplicated falciparum malaria in pediatric patients in Gabon. *J. Infect. Dis.* **198**, 911–919 (2008).
45. Vivas, L. *et al.* Anti-malarial efficacy of pyronaridine and artesunate in combination *in vitro* and *in vivo*. *Acta Trop.* **105**, 222–228 (2008).
46. Ringwald, P., Bickii, J. & Basco, L. Randomised trial of pyronaridine versus chloroquine for acute uncomplicated falciparum malaria in Africa. *Lancet* **347**, 24–28 (1996).
47. Carr, A. & Amin, J. Efficacy and tolerability of initial antiretroviral therapy: a systematic review. *AIDS* **23**, 343–353 (2009).
48. Grant, A., Gothard, P. & Thwaites, G. Managing drug resistant tuberculosis. *BMJ* **337**, a1110 (2008).
49. Lawpoolsri, S. *et al.* Optimally timing primaquine treatment to reduce *Plasmodium falciparum* transmission in low endemicity Thai-Myanmar border populations. *Malar. J.* **8**, 159 (2009).
50. Vale, N., Moreira, R. & Gomes, P. Primaquine revisited six decades after its discovery. *Eur. J. Med. Chem.* **44**, 937–953 (2009).
51. Cappellini, M. D. & Fiorelli, G. Glucose-6-phosphate dehydrogenase deficiency. *Lancet* **371**, 64–74 (2008).

52. [No authors listed]. *Roll Back Malaria Partnership. Global Malaria Action Plan for a malaria-free world* [online] (2009).
53. Samarasekera, U. Countries race to contain resistance to key antimalarial. *Lancet* **374**, 277–280 (2009).
54. Maude, R. J. *et al.* The last man standing is the most resistant: eliminating artemisinin-resistant malaria in Cambodia. *Malar. J.* **8**, 31 (2009).
Modelling paper showing that the absolute number of artemisinin-resistant malaria cases will decrease, but the proportion of artemisinin-resistant malaria cases will increase over time when elimination of resistant malaria is attempted.
55. Korsinczky, M. *et al.* 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 (2000).
56. Okell, L. C., Drakeley, C. J., Bousema, T., Whitty, C. J. & Ghani, A. C. Modelling the impact of artemisinin combination therapy and long-acting treatments on malaria transmission intensity. *PLoS Med.* **5**, e226 (2008).
57. Castle, S. J., Toscano, N. C., Prabhaker, N., Henneberry, T. J. & Palumbo, J. C. Field evaluation of different insecticide use strategies as resistance management and control tactics for *Bemisia tabaci* (Hemiptera: Aleyrodidae). *Bull. Entomol. Res.* **92**, 449–460 (2002).
58. Boni, M. F., Smith, D. L. & Laxminarayan, R. Benefits of using multiple first-line therapies against malaria. *Proc. Natl Acad. Sci. USA* **105**, 14216–14221 (2008).
Modelling paper showing the delay in development of antimalarial drug resistance with regional deployment of MFTs.
59. Shretta, R., Omumbo, J., Rapuoda, B. & Snow, R. W. Using evidence to change antimalarial drug policy in Kenya. *Trop. Med. Int. Health* **5**, 755–764 (2000).
60. Coatney, G. R. Pitfalls in a discovery: the chronicle of chloroquine. *Am. J. Trop. Med. Hyg.* **12**, 121–128 (1963).
61. Jensen, M. & Mehlhorn, H. Seventy-five years of Resochin in the fight against malaria. *Parasitol. Res.* **105**, 609–627 (2009).
62. Greenwood, B. M. *et al.* Malaria: progress, perils, and prospects for eradication. *J. Clin. Invest.* **118**, 1266–1276 (2008).
63. Payne, D. Spread of chloroquine resistance in *Plasmodium falciparum*. *Parasitol.Today* **3**, 241–246 (1987).
64. Djimde, A. *et al.* A molecular marker for chloroquine-resistant falciparum malaria. *N. Engl. J. Med.* **344**, 257–263 (2001).
65. Fidock, D. A. *et al.* Mutations in the *P. falciparum* digestive vacuole transmembrane protein PfCRT and evidence for their role in chloroquine resistance. *Mol. Cell* **6**, 861–871 (2000).
66. Wootton, J. C. *et al.* Genetic diversity and chloroquine selective sweeps in *Plasmodium falciparum*. *Nature* **418**, 320–323 (2002).
67. Kublin, J. G. *et al.* Reemergence of chloroquine-sensitive *Plasmodium falciparum* malaria after cessation of chloroquine use in Malawi. *J. Infect. Dis.* **187**, 1870–1875 (2003).
68. Laufer, M. K. *et al.* Return of chloroquine antimalarial efficacy in Malawi. *N. Engl. J. Med.* **355**, 1959–1966 (2006).
69. Plowe, C. V. The evolution of drug-resistant malaria. *Trans. R. Soc. Trop. Med. Hyg.* **103**, S11–S14 (2009).

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Competing interests statement
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DATABASES
Entrez Genome Project:
http://www.ncbi.nlm.nih.gov/sites/entrez?db=genomeprj
Plasmodium falciparum
Entrez Gene: http://www.ncbi.nlm.nih.gov/entrez
pfcrt | *pfmdr1*

FURTHER INFORMATION
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