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Artemisinin-based combination therapies: a vital tool in efforts to eliminate malaria

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Abstract

Plasmodium falciparum resistance to chloroquine and sulphadoxine–pyrimethamine has led to the recent adoption of artemisinin-based combination therapies (ACTs) as the first line of treatment against malaria. ACTs comprise semisynthetic artemisinin derivatives paired with distinct chemical classes of longer acting drugs. These artemisinins are exceptionally potent against the pathogenic asexual blood stages of *Plasmodium* parasites and also act on the transmissible sexual stages. These combinations increase the rates of clinical and parasitological cures and decrease the selection pressure for the emergence of antimalarial resistance. This Review article discusses our current knowledge about the mode of action of ACTs, their pharmacological properties and the proposed mechanisms of drug resistance.

Six decades ago, hopes of malaria eradication were raised by the discovery and implementation of chloroquine (CQ). The use of this highly effective, fast-acting and inexpensive 4-aminoquinoline, along with the potent insecticide dichlorodiphenyltrichloroethane (DDT), quickly proved successful in substantially reducing the incidence and severity of malaria worldwide. Initial successes were achieved primarily in regions with temperate climates and seasonal malaria transmission. However, in many parts of the world eradication efforts were effectively thwarted by multiple issues, including insecticide resistance in *Anopheles* mosquito vectors, high rates of *Plasmodium* parasite transmission, logistical hurdles to implementing

DATABASES

Entrez Genome Project: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=genomeprj
Anopheles gambiae | *Plasmodium berghei*|
Plasmodium chabaudi | *Plasmodium falciparum* |
Plasmodium vivax | Saccharomyces cerevisiae | Xenopus laevis
UniProtKB: http://www.uniprot.org
PfATP6 | PfCRT | PfMDR1 | PfMRP | UBP-1

FURTHER INFORMATION

David A. Fidock's homepage: http://microbiology.columbia.edu/fidock/Fidocklab/Fidock_lab_home.html
Affordable Medicines Facility – malaria: http://www.theglobalfund.org/en/amfm
Bill and Melinda Gates Foundation: http://www.gatesfoundation.org/Pages/home.aspx
The Global Fund to Fight AIDS, Tuberculosis and Malaria: http://www.theglobalfund.org/en
Medicines for Malaria Venture: http://www.mmv.org
President's Malaria Initiative: http://www.fightingmalaria.gov
Roll Back Malaria Partnership: http://www.rollbackmalaria.org
World Bank: http://www.worldbank.org
WWARN: http://www.wwarn.org

control strategies, wars and population displacements, and a lack of sustained funding. Subsequently, resistance to CQ and the cost-effective replacement drug sulphadoxine–pyrimethamine (SP) emerged in the most lethal human malarial pathogen, *Plasmodium falciparum*[1,2]. In some areas, the switch to either mefloquine (MFQ) or quinine resulted in the appearance of multidrug-resistant parasites, particularly in Southeast Asia[3]. The global consequence was a resurgence of malaria morbidity and mortality. With nearly 40% of the global population at risk, 300–660 million episodes of clinical *P. falciparum* malaria occur annually and there are an estimated one million deaths[4]. Most of these occur in sub-Saharan Africa (FIG. 1a), where rates of transmission can reach 1,500 mosquito-delivered parasite inoculations per individual per year[5].

Now there is hope that the tide may turn again with the implementation of artemisinin-based combination therapies (ACTs). Their success in treating CQ- and SP-resistant malaria has prompted the WHO to recommend ACTs as the preferred first-line antimalarials against *P. falciparum* malaria and has elicited substantial funding and logistical support from, among others, The Global Fund to Fight AIDS, Tuberculosis, and Malaria, The World Bank, Roll Back Malaria, the President's Malaria Initiative, the Medicines for Malaria Venture, and the Bill and Melinda Gates Foundation. ACTs have now been widely adopted across sub-Saharan Africa (FIG. 1b). Their outstanding efficacy, together with complementary interventions that reduce transmission, such as long-lasting insecticide-treated bed nets and indoor residual insecticide spraying, has led to a renewed call for eradicating malaria[6].

Is eradication, or even progressive elimination, feasible? To begin to address this, we review artemisinin (ART) derivatives and their partner drugs in terms of their modes of action, pharmacokinetic properties and proposed mechanisms of antimalarial resistance. We also refer to therapeutic strategies that might decrease the emergence of drug resistance and, finally, we present a perspective on the current ACT-based efforts to reduce the burden of malaria.

ARTs

Discovery and synthesis

A major advance in the search for effective treatments for drug-resistant malaria came with the isolation of ART (also known as qinghaosu) from the Chinese wormwood *Artemisia annua*[7] (Supplementary information S1 (figure)). This sesquiterpene lactone endoperoxide is extremely potent against CQ- and SP-resistant *P. falciparum in vitro* and *in vivo* and can produce faster parasite clearance and fever resolution times than any other licensed antimalarial, including quinine[8]. However, ART must first be extracted and then chemically modified to produce semisynthetic derivatives with improved pharmacological properties (see below), which adds significantly to the cost of ACT therapy. As an alternative, *Saccharomyces cerevisiae* has been engineered to synthesize artemisinic acid, a precursor of ART[9]. This scalable production system has the potential to reduce the cost of goods and ensure a steady supply of the drug, thereby possibly alleviating two major concerns as the world transitions to first-line therapy with ACTs.

Artemisinin derivatives

Current ACTs use the ART derivatives artemether (ATM), artesunate (AS) or dihydroartemisinin (DHA) (Supplementary information S1 (figure)), which have improved oral bioavailability compared with that of ART. In humans, these derivatives rapidly achieve peak plasma levels and possess elimination half-lives of approximately 1–3 hrs[8,10] (TABLE 1). Their metabolism is partly mediated by the cytochrome P450 enzymes CYP2B6 and CYP3A4 (REF. [10]). Bioconversion of ATM and AS produces DHA along with other metabolites.

Mechanisms of action and parasite resistance

The mechanisms by which ARTs exert their antimalarial activity remain contentious[11]. Nevertheless, most studies concur that the activity of ART and many if not all of its potent derivatives results from reductive scission of the peroxide bridge by reduced haem iron, which is produced inside the highly acidic digestive vacuole (DV) as it digests haemoglobin. In support of this, a recent study with fluorescent ART trioxane derivatives provided evidence for their rapid accumulation in the DV and their activation by neutral lipid-associated haem[12]. Other studies with fully synthetic endoperoxides (including trioxolanes and trioxaquines) as well as ART found that these compounds can alkylate haem, both *in vitro* with *P. falciparum* and *in vivo* in rodent malaria models, and identified a correlation between the degree of alkylation and the potency of trioxolanes against cultured *P. falciparum* asexual blood-stage parasites[13–16]. In addition to the formation of potentially toxic haem-adducts, activated ART (for which the site of action remains unclear; FIG. 2) might in turn generate free radicals that alkylate and oxidize proteins and possibly lipids in parasitized erythrocytes[17–19]. In agreement with this, ART activity can be potentiated by oxygen and oxidizing agents and attenuated by reducing agents[17]. Alternatively, ART derivatives might be activated by peroxide bond cleavage by intracellular iron–sulphur redox centres, which are found in multiple *Plasmodium* spp. enzymes. Subsequent alkylation of these iron–sulphur-containing enzymes could result in parasite death[20]. A role for non-haem iron sources in the activation of ART is supported by the antagonism between multiple endoper-oxides (including ART) and chelators that are specific to nonhaem iron[21]. Recent transmission electron microscopy studies also indicate that ART and other endoperoxides can compromise the integrity of the DV in a dose- and time-dependent manner, before any effect is observed on the endoplasmic reticulum and the mitochondria[22].

Investigations into potential protein targets of ARTs have included studies with radiolabelled ART that identified several covalently modified proteins, suggesting that their alkylation and inactivation might account for parasite death[23,24]. Separate studies have focused on PfATP6, a *P. falciparum* SERCA-type calcium-dependent ATPase[25] in the endoplasmic reticulum. In transfected *Xenopus laevis* oocytes, PfATP6 activity is abolished by ART but is unaffected by other antimalarials, including the inactive compound desoxyartemisinin, which lacks the endoperoxide bridge[25]. Single nucleotide polymorphisms (SNPs) in *pfatp6* were observed in some *P. falciparum* isolates from French Guiana and Senegal that displayed reduced *in vitro* susceptibility to ARTs, suggesting a causal association[26]. However, a recent analysis of *pfatp6* sequence conservation in 388 clinical isolates from 17 different countries identified 33 SNPs, 29 of which were non-synonymous mutations, implying a high degree of genetic diversity[27]. A direct association between *pfatp6* SNPs and decreased ART susceptibility has yet to be experimentally documented in *P. falciparum.*

Studies with transgenic *P. falciparum* asexual blood-stage parasites cultured *in vitro* have found that ART susceptibility can be influenced by genetic changes in the loci encoding *P. falciparum* multidrug resistance protein 1 (PfMDR1; also known as Pgh-1) and *P. falciparum* chloroquine resistance transporter (PfCRT). Point mutations in both genes, as well as *pfmdr1* gene duplications, are known to affect parasite responses to diverse antimalarials. The potencies of these drugs can be altered by their degree of accumulation inside the DV, which is the site of haem detoxification[28]. Both of these putative transporters are located on the membrane of the DV in intra-erythrocytic parasites, suggesting that they are important regulators of drug accumulation in the DV. An association between increased *pfmdr1* copy number and a higher risk of treatment failure using AS paired with MFQ has been observed in a clinical study conducted in Thailand[29]. Transfection studies have since confirmed this association between *pfmdr1* amplification and reduced susceptibility to ART and MFQ[30]. Recent gene disruption evidence now suggests that *P. falciparum* multidrug resistance-

associated protein 1 (PfMRP1), which resides on the parasite plasma membrane, can additionally influence ART susceptibility *in vitro*[31].

Now, evidence has emerged of decreased ART efficacy in *P. falciparum* isolates from Thailand and Cambodia[32,33]. This has raised concerns of a potentially imminent spread of ART resistance, especially given that both CQ and SP resistance emerged first in Southeast Asia and subsequently migrated to Africa[34,35]. This pattern of migration might reflect the substantial drug pressure that is applied to malarial infections in Southeast Asia, where hosts have minimal immunity and there is little competition between parasite clones *in vivo* because of the low rates of transmission (BOX 1). This would favour the dissemination of drug-resistant parasites even if they harboured mutations that conferred a fitness cost, because they would rarely encounter drug-sensitive parasites in the same host and would be frequently subjected to drug pressure[36]. Southeast Asian parasites might also have a particular capacity to hypermutate in response to drug pressure[37]. Clinical groups are currently documenting cases of delayed parasite clearance times in patients who were given ART monotherapy or ACTs. Genomic analysis of such isolates, using the recently developed methods of rapid genome sequencing, haplotype maps and genome tiling arrays, should yield candidate genes that can be evaluated using transfection-based approaches[38–40]. In a separate approach using the rodent parasite model *Plasmodium chabaudi*, researchers have now selected genetically stable parasite lines that are resistant to ART and AS[41]. These parasites lacked mutations in the *P. chabaudi* homologues of *pfatp6* and *pfmdr1*. Further genetic analysis identified a SNP in each of the resistant clones in the gene that encodes a putative deubiquitinating protease termed UBP-1 (REF. [42]). Such studies with human parasites and rodent models should enable the identification of molecular markers that can be used to track the emergence and spread of *P. falciparum* parasites with reduced ART susceptibility.

Box 1

A complex disease

One of the main challenges that affects malaria control efforts is the diversity of epidemiological contexts in different malaria-endemic regions. One key variable involves disease transmission. The entomological inoculation rate (that is, the number of infectious bites per person per year) varies from 1 to 2 in many parts of Southeast Asia up to as high as 1,500 in some settings in Africa[115]. Factors that influence this rate include human proximity to mosquito larval habitats[116], the local mosquito vector (*Anopheles gambiae* is the main African vector and is widely regarded as the most dangerous because of its vector competence, singular preference for humans over other animals and prolific breeding[117]) and environmental conditions (including temperature, altitude, humidity and patterns of seasonal rainfall[118]).

Differences in the transmission and endemicity in malaria-endemic regions have a direct influence on clinical outcomes of infection. Owing to the low transmission rate in Southeast Asia, most infections are symptomatic and therefore treated with antimalarials. This is in contrast to Africa, where more frequent transmission leads to the acquisition of immunity. However, this immunity is not sterilizing and typically manages to only lower the parasite burden and reduce the clinical impact. Immunity acquired in Africans who live in areas of moderate to high levels of transmission is referred to as premunition. This refers to an immune state that is contingent on chronic infection and that rapidly wanes if the resident moves to a non-malarious region for a period of months to years[119]. As a result of the partial immunity that typically develops in repeatedly infected Africans, many cases of malaria on that continent are asymptomatic and are therefore not treated with antimalarials[120]. This in turn substantially influences the dynamics of intrahost parasite competition and the spread of drug resistance[121].

In addition to mosquito, environmental and parasite factors, host factors affect both the burden and severity of disease. For a discussion of the key host factors, readers are directed to recent comprehensive reviews[122–124].

ACTs

The poor pharmacokinetic properties of ART and its derivatives, including the short half-lives of this chemical class, translate into substantial treatment failure rates when used as monotherapy[43]. Combining a member of this class with a longer-lasting partner drug assures sustained anti-malarial pressure after the plasma concentrations of the ART derivatives have fallen below therapeutic levels. This increases the antimalarial treatment efficacy and reduces the selective pressure for resistance. Ideally, antimalarial combination drug partners would have similar pharmacokinetic properties so that no drug is left 'unprotected' by the other. That said, ACTs benefit substantially from the ability of the ART derivative to rapidly reduce the parasite biomass, resulting in few parasites to be cleared by the partner drug and reducing the pool of parasites from which resistance can emerge. Below, we present the key partner drugs that are currently in use. In addition, SP has also been used in combination with the derivative AS[44]. However, this combination is not reviewed, owing to the increasing prevalence of SP-resistant parasites that reduce treatment efficacy and the availability of a recent and comprehensive review[2].

Mefloquine

MFQ, a fluorinated 4-quinoline, is moderately well absorbed orally and possesses an elimination half-life of 2–3 weeks, partly owing to its high lipophilicity and extensive tissue distribution. Earlier work showed that MFQ associates with intra-erythrocytic haemozoin[45]. However, evidence that this association might be secondary to a primarily cytosolic mode of action comes from studies with transgenic *P. falciparum* lines expressing different *pfmdr1* copy numbers, which observed that reduced parasite susceptibility to MFQ (and ART) was associated with increased PfMDR1-mediated solute import into the DV[46]. As alluded to earlier, human clinical data, as well as *in vitro* studies, reveal that *pfmdr1* gene amplification is a major determinant of MFQ resistance and is associated with an increased risk of treatment failure and recrudescence with MFQ monotherapy or AS–MFQ combination therapy[29,47]. Point mutations in *pfmdr1* can also alter *P. falciparum* susceptibility to MFQ *in vitro*, although this does not seem to substantially affect the treatment outcome[29].

MFQ was first introduced as a first-line antimalarial in Thailand in the mid 1980s, but resistance emerged in a few years[3]. Therefore, in 1994 MFQ was paired with AS (AS–MFQ) as a 3-day combination regimen. The main clinical benefits of AS–MFQ were made evident by its 100% cure rate measured in 1998, versus a cure rate of 71% with MFQ monotherapy measured in 1990 (REF. [48]). Further investigations into the pharmacokinetic–pharmacodynamic properties of AS–MFQ are required to better understand why this combination has remained so effective, even against highly MFQ-resistant parasites[49,50].

Lumefantrine

Lumefantrine (LMF; also called benflumetol) is structurally related to the hydrophobic arylamino-alcohol antimalarials, including MFQ, quinine and halofantrine (Supplementary information S1 (figure)), suggesting that they have similar modes of action[51]. The selection of ATM and LMF as a combination (known as Coartem; Novartis/Chinese Academy of Medical Military Sciences) stems in part from their reported synergistic effects against *P. falciparum in vitro*[52]. The 3-day, 6-dose ATM–LMF regime has proven highly effective in treating *P. falciparum* infections[50] and is prioritized by the WHO as a replacement for CQ and SP monotherapies. The pharmacokinetic properties of LMF include a large apparent volume of

distribution and a terminal elimination half-life of 4–5 days[53]. Human pharmacokinetic and pharmacodynamic studies correlate the risk of clinical failure (the likelihood to recrudesce) with plasma LMF concentrations falling below 280 ng per ml[54]. These findings, combined with the report of up to 15-fold variability in LMF plasma concentrations in clinical trial volunteers[55], highlight the importance of appropriate dosing with LMF-containing combinations.

There has been considerable debate about the probability of parasites developing resistance to LMF in Africa. Some suggest that the emergence of resistance will be slower in Africa than in Southeast Asia because of the intrinsic differences of the parasites in intrahost parasite competition, drug exposure and host immunity (BOX 1). Alternatively, the high transmission rates in Africa and subsequent exposure of many parasites to subtherapeutic concentrations of this drug might repeatedly select for the emergence and subsequent dissemination of resistant parasites[56]. *In vitro* investigations have reported some cross-resistance between LMF and MFQ and have identified copy number changes and SNPs in *pfmdr1* that can decrease parasite susceptibility to both agents[30,57]. ATM–LMF treatment failure has been associated in Asia with *pfmdr1* amplification and in Africa with selection for certain PfMDR1 polymorphic residues (Asn86, Phe184 and Asp1246)[57–59]. In a study from Zanzibar, selection for *pfmdr1* variants seemed to be operating mainly in re-infections that occurred during the elimination phase of LMF[60]. Recent clinical and *in vitro* studies have also observed that mutant *pfcrt* alleles that confer CQ resistance can enhance susceptibility of the parasite to LMF and ART[61]. These findings are consistent with the excellent clinical efficacy of this combination in regions where CQ resistance remains highly prevalent. In 2008, Coartem was used to treat 70 million cases of malaria, most of which were in Africa. A dispersible formulation of Coartem has also been developed for babies and children through a partnership between Novartis and the Medicines for Malaria Venture. This dispersible formulation has been found to be highly effective, achieving a 98% clinical cure rate in a large study involving several African countries[62]. It has a sweet fruit flavour and costs under US$0.40 per child for public-sector buyers, making this an attractive ACT for the treatment of malaria in children.

Amodiaquine

Amodiaquine (AQ), a potent 4-amino-quinoline antimalarial, is used infrequently because of reported toxicity. This toxicity includes instances of drug-induced agranulocytosis and hepatitis, which are thought to result from AQ bio-activation to a protein-reactive quinoneimine metabolite[63]. These adverse events, however, were generally associated with the use of AQ as a prophylactic. A meta-analysis of clinical studies found that therapeutic AQ regimes with a total dose of up to 35 mg per kg body weight over 3 days were as well tolerated as CQ or SP for the treatment of uncomplicated *P. falciparum* malaria[64]. However, one recent study reported that an increased risk of neutropenia was associated with AQ therapy in patients with HIV who were receiving antiretroviral therapy[65].

In vivo AQ is rapidly converted by hepatic P450 enzymes into monodesethyl-AQ. This metabolite, which retains substantial antimalarial activity, has a half-life in blood plasma of 9–18 days and reaches a peak concentration of ~500 nM 2 hours after oral administration. By contrast, AQ has a half-life of ~3 hours, attaining a peak concentration of ~25 nM within 30 minutes of oral administration[66]. *In vivo* clearance rates of AQ, however, display a variation between individuals that ranges from 78 to 943 ml per minute per kg[67]. One study recently reported an increased risk of treatment failure with parasites that had *in vitro* monodesethyl-AQ half-maximal inhibitory concentration (IC_{50}) values of above 60 nM[68].

AQ is structurally closely related to CQ and might also prevent haem detoxification (see below). Indeed, AQ has been reported to interact with μ-oxo dimers of haem ((FeIII-protoporphyrin IX)$_2$O) *in vitro*, although this has not been confirmed with cultured

parasites[69]. PfCRT Lys76Thr and PfMDR1 Asn86Tyr substitutions are both associated with decreased susceptibility to AQ and CQ, and CQ-resistant parasites can have reduced accumulation of AQ[70–72]. However, both *in vitro* and *in vivo* evidence suggest that cross-resistance between the two drugs is incomplete and that AQ can remain effective against some CQ-resistant parasites[64,73]. Analysis of the selective pressure on *pfmdr1* before and after treatment with AQ found a positive selection for the Asn86Tyr mutation, contrasting with selection against this mutant allele following ATM–LMF treatment[58,59,74].

Currently, AQ is partnered with AS and is available as fixed-dose tablets. Clinical investigations have shown AS–AQ to be an effective ACT with an acceptable safety profile, with the exception of infrequent instances of drug-induced anaemia[75]. In a comparative ACT trial in Angola, AS–AQ resulted in a lower level of gametocytes than ATM–LMF[76]. However, a clinical trial in Papua, Indonesia, found that AS–AQ-treated patients with mixed *P. falciparum* and *Plasmodium vivax* infections had a higher parasitological failure rate, gametocyte carriage and risk of anaemia than those treated with DHA and piperaquine (PQP)[77].

Piperaquine

PQP is a bisquinoline and is also structurally related to CQ. This potent and well-tolerated drug was adopted as the primary antimalarial in China during the 1970s and 1980s in response to increasing CQ treatment failure rates[78]. Although studies on the mode of action of PQP in *P. falciparum* remain limited, investigations in the rodent parasite *Plasmodium berghei* have found that this drug acts mainly on mature asexual blood-stage trophozoites[79]. PQP is postulated to accumulate in the DV and bind to haem-containing species, inhibiting haem detoxification. PQP-treated *P. berghei* parasites have swollen DVs and abnormal haemozoin clumping[80]. PQP is currently being evaluated in combination with DHA (DHA–PQP). Pharmacologically, PQP is characterized by a large volume of distribution and reduced rates of excretion after multiple doses. This lipophilic drug is rapidly absorbed, with a T_{max} (time to reach the highest concentration) of 2 hours after a single dose[81]. In clinical trials, the cure rates, fever and parasite clearance times[82,83] of DHA–PQP were similiar to those of AS–MFQ. DHA–PQP was also better tolerated, with no clinically significant cardiovascular or metabolic effects[84]. One concern was that the percentages of circulating erythrocytes that harboured gametocytes were reportedly higher in patients who received DHA–PQP than in those treated with AS–MFQ, suggesting that DHA–PQP may not be as effective in reducing parasite transmission to mosquito vectors as other combinations[83,85].

PQP-resistant *P. falciparum* has been reported in China, presumably as a result of its one-time widespread use as monotherapy and perhaps worsened by its long elimination half-life (~5 weeks)[80]. Debate continues on the genetic basis of PQP resistance and the extent to which this affects CQ susceptibility[86,87]. Clarifying this will provide important biomarkers of resistance to monitor clinical DHA–PQP efficacy.

Pyronaridine

Pyronaridine (PYR) is an acridine-type (benzonaphthyridine) Mannich base. It was first synthesized in China and was introduced as a new anti-malarial drug in 1970. PYR is highly potent against *P. falciparum*, with mean fever subsidence times of 1–2 days and parasite clearance times of 2–3 days. PYR has an excellent therapeutic index in treating mice infected with *P. berghei*[88]. PYR activity is limited to the asexual blood stages, with no apparent efficacy against the preceding asymptomatic liver stages or the intra-erythrocytic gametocyte forms[89,90].

PYR inhibits the formation of β-haematin (a synthetic form of haemozoin[91]) *in vitro*, forms complexes with haematin, inhibits glutathione-dependent degradation of haem-containing species and enhances haematin-induced lysis of infected erythrocytes[92]. Furthermore, treatment of *P. falciparum* and *P. berghei* with PYR leads to rapid changes in the morphology of their DV[93]. Additional morphological changes include the formation of multilamellate whorls, swelling of the pellicular complexes and distended and granulated mitochondria[93]. These effects were also observed in a PYR-treated CQ-resistant line of *P. berghei*, whereas the DV showed no significant change, suggesting that PYR might also possess a secondary DV-independent mode of action[93].

In China, clinical trials against both CQ-sensitive and CQ-resistant *P. falciparum* infections found PYR monotherapy to be highly effective when administered orally over 2–3 days[94]. In a trial in Cameroon, PYR was 100% effective at eliminating malarial symptoms after 3 days, versus 44% efficacy for CQ[95]. However, patient follow-up extended to only 14 days, and a subsequent trial that examined infections that occur more than 28 days after treatment observed a small percentage of recrudescent infections[96]. AS–PYR has recently completed several large-scale Phase III clinical trials, and initial results demonstrate excellent efficacy[97] (I. Borghini-Fuhrer, personal communication).

Artemisinin-based combination therapy efficacy against *P. vivax*

ACTs also constitute an excellent treatment for *P. vivax*, the cause of benign tertian malaria and an important malarial pathogen with highest prevalence outside Africa[98]. In one trial in Papua, Indonesia, DHA–PQP and ATM–LMF were both highly effective in resolving uncomplicated *P. vivax* malaria, reducing anaemia and decreasing the number of gametocytes[99]. Late relapses were attributed to the emergence of previously dormant hypnozoites from the liver, a biological characteristic of *P. vivax* that is not shared by *P. falciparum* and that is only responsive to primaquine treatment. AS–PYR was also reported to be highly effective in treating *P. vivax* malaria, with similar cure rates and faster parasite and fever clearance times than those of those of CQ (which continues to be widely used to treat *P. vivax* malaria)[100].

Artemisinin toxicity concerns

Earlier animal studies with ARTs had raised concerns about toxicity, including reports of increased embryo lethality or malformations early post-conception in pregnant rats and rabbits[101]. Studies in dogs, rats and monkeys also found that ARTs could cause occult brainstem neurotoxicity[102]. As a precaution, the WHO does not recommend ACT treatment for children weighing ≤5 kg or women in their first trimester of pregnancy. Clinical evidence nevertheless supports the safety of ACTs in children weighing ≥5 kg and pregnant women in subsequent trimesters. One prospective study in a cohort of nearly 500 pregnant women with acute *P. falciparum* malaria indicated that ARTs were well tolerated during pregnancy, including during the first trimester, with no evidence of adverse effects[103]. Birth outcomes among treated individuals did not differ greatly from community rates for abortion, stillbirth, congenital abnormality and mean gestation at delivery. A more recent study with 50 pregnant women treated with DHA–PQP found no serious adverse events and no evidence of toxicity for either the fetus or the mother[104]. Additional studies are required to further evaluate the safety of ARTs and the principal ACTs in these populations[105].

Further issues for ACT implementation

The application of ACTs to combat CQ- and SP-resistant *P. falciparum* malaria has produced demonstrable improvements in morbidity and mortality rates in Africa[106]. Several examples of this are illustrated in FIG. 3. In addition to parasite resistance, multiple other factors affect

the effectiveness of these ACTs, especially their pharmacokinetic properties. A notable example is LMF, the clinical cure rates of which were found to significantly improve when the drug was administered with a high fat meal[54], and ATM–LMF efficacy was found to be dramatically enhanced using a six-dose regimen as opposed to a four-dose regimen[57].

As ACTs become the first-line antimalarial therapy, increased selection pressure will be placed on this valuable resource. Comprehensive and standardized monitoring programmes will be essential to assess therapeutic efficacies, determine drug susceptibilities *in vitro*, screen for resistance markers and distinguish recrudescences from reinfections in treated patients[107, 108]. These programmes should benefit from the WorldWide Antimalarial Resistance Network (WWARN), which was established to coordinate antimalarial resistance monitoring[109]. Maximizing the efficacy and longevity of ACTs will require the continued development of supply and distribution infrastructures, adequate health care worker training in ACT usage, sustained financial support of implementation programmes and a detailed understanding of antimalarial efficacy.

However, the prevalence of counterfeit or clinically substandard ACTs that contain small quantities of the ART derivative threatens to subvert ACT efficacy. This provides an ideal mechanism for the selection of resistance. Recent estimates are that 33%–53% of all ACT tablets in mainland Southeast Asia are counterfeit[110]. One study carried out in six African countries also documented substandard medicine in 35% of ACTs purchased from private pharmacies and found ART monotherapy to be common despite the appeal by the WHO in January 2006 to halt its production[111]. In addition, although ACTs have clearly proven effective for the treatment of malaria in Southeast Asia, where transmission is typically low, concerns remain about their long-term implementation as first-line therapy in high-transmission areas in Africa. This is exacerbated by the crucial dependence on ART derivatives in virtually all of the existing antimalarial combination therapies, creating a substantial selection advantage for parasites with decreased susceptibility to ARTs. Evidence for the emergence of these parasites in parts of Southeast Asia[32,33] necessitates urgent containment measures and underscores the need for the continual development of alternative antimalarials[112] and new treatments using existing drugs (BOX 2).

Box 2

Drug rotations and chemosensitizing agents

If faced with the spread of artemisinin-resistant *Plasmodium falciparum*, one potential strategy could be to introduce chloroquine (CQ)-containing combinations that maintain efficacy against CQ-resistant strains. CQ is inexpensive and has a good safety profile. It has a well-characterized mode of action (inhibiting haem detoxification by binding to haemozoin, an immutable host factor[51,91]), and resistance to CQ has been clearly attributed to multiple point mutations in the *P. falciparum* CQ resistance transporter (PfCRT)[125, 126]. The PfCRT Lys76Thr mutation in particular has proved to be a highly sensitive molecular marker of CQ resistance, providing an effective epidemiologic tool to assess CQ efficacy[28]. Recent studies in Malawi showed that the absolute removal of CQ for a decade led to the virtual disappearance of parasite strains harbouring mutant *pfcrt* and restoration of CQ clinical efficacy to >98% (REF. [127]). This has been attributed to a fitness cost associated with the Lys76Thr mutation, as recovery of the CQ-sensitive phenotype was associated with the expansion of wild-type *pfcrt* from the endogenous population rather than a genetic reversion[128]. Decreases in the prevalence of parasites possessing PfCRT Lys76Thr have also been observed in Gabon, Vietnam, China and Thailand[129]. Resistance reversal agents would potentially reduce the large selection pressure that CQ would exert on parasite reservoirs with mutant *pfcrt* alleles. One such agent is the antihistamine chlorpheniramine, which has been demonstrated clinically to ameliorate CQ efficacy[130].

Indeed, a recent study found comparable mean fever clearance times between groups treated with CQ–chlorpheniramine or amodiaquine partnered with sulphadoxine–pyrimethamine (AQ–SP). However, CQ–chlorpheniramine produced delayed parasite clearance times and increased failure rates relative to those of AQ–SP[131]. More potent antimalarials with reversal properties will need to be identified for this strategy to be widely implemented.

The eradication of malaria?

The success of the ACTs has led to a new call for the eradication of malaria. Is this possible? The introduction of CQ and DDT once raised hopes of malaria eradication, and substantial gains were made throughout Asia and South America. However, it was quickly realized that elimination of the disease in Africa was not achievable at the time.

Now, malaria control and progressive elimination is moving up the political agenda, led in no small part by the Bill and Melinda Gates Foundation. This has caused economists to present cogent arguments regarding the cost-effectiveness of tackling malaria. The means to do this are available in the form of vector control (including the use of long-lasting insecticide-treated bed nets and indoor residual spraying; BOX 3), intermittent preventive treatment in pregnancy and, increasingly, infancy, (BOX 4) and early diagnosis and treatment with effective ACTs. This has once again raised the possibility of eradication. In October 2007, Bill and Melinda Gates called on global leaders to embrace “an audacious goal — to reach a day when no human being has malaria, and no mosquito on earth is carrying it.” This goal has been enthusiastically supported by the director general of the WHO, Dr Margaret Chan. UN Secretary-General Ban Ki-moon called for “ensuring universal coverage by the end of 2010”, supported by the Roll Back Malaria Partnership. The substantial commitment and financial infusion is already providing beneficial results for those living in malaria-endemic regions (FIG. 3). However, the difficult task remains of acquiring, distributing and implementing the tools that are required to reduce malaria-related morbidity and mortality and interrupt disease transmission[113] — namely ACTs, vector control measures and effective education of community health workers.

Box 3

Vector control measures

Alongside chemotherapeutic interventions, *Anopheles* vector control programmes are an essential component of efforts to decrease the malaria burden. These include the use of long-lasting insecticide-treated bed nets and insecticides to interrupt transmission. Currently, only pyrethroid insecticides are approved for treating bed nets. Careful monitoring of insecticide-resistant mosquitoes is necessary to ensure continued effective- ness. In addition, indoor residual spraying (using either dichlorodiphenyltrichloroethane (DDT) or pyrethroid insecticides) is a key vector control method that has been proven to decrease malaria transmission and reduce the risk of malaria-related illness and death[132].

Box 4

Intermittent preventive treatment

The WHO advocates intermittent preventive treatment (IPT) in areas with a high prevalence of malaria as a means to prevent or reduce the adverse outcomes that are associated with malarial infection during pregnancy[133,134]. This involves the treatment of asymptomatic pregnant women, regardless of their parasite infection status, with regularly spaced therapeutic doses. Currently the only antimalarial approved for IPT during pregnancy (IPT_p) is sulphadoxine–pyrimethamine (SP), which is also the primary antimalarial used

for IPT of infants (IPTi). This is mainly due to the limited safety, toxicology and efficacy data of other antimalarials in these highly vulnerable patient populations. The benefit of IPT stems from the clearance or suppression of asymptomatic infections combined with a prophylactic effect during the long drug elimination phase. IPTp clinical studies recently observed that among HIV-negative women, a two-dose regimen of SP led to a reduction in the risk of placental malaria, low birth weight and maternal anaemia compared with risks in women given a placebo[135]. To achieve similar benefits in HIV-positive women required more frequent dosing[135]. Studies in Tanzania, Ghana and Mozambique have demonstrated that IPTi also led to a significant decrease in the incidence of cases of malaria in children[136–138].

The major concern with SP-based IPT_p and IPTi is the increasing prevalence of SP-resistant parasites, which decreases the treatment and prophylactic efficacy[139]. Therefore, there is an urgent need to identify replacements for SP. Ideal candidates should have a long half-life (as current evidence suggests that prophylaxis might be the most important determinant of IPT efficacy), be well tolerated to ensure a high degree of compliance, be easy and safe to administer during pregnancy and be inexpensive.

A key part of the strategy to decrease the malaria burden is to ensure universal access to ACTs. Although subsidized antimalarials are currently available through public facilities in most malaria-endemic areas, only a percentage of these populations has access to these sources, and those ACTs available through the private sector are priced according to market demands. To address this issue, the AMFm (Affordable Medicines Facility — malaria) has been formed to increase patient access to ACTs, following a recommendation from the US Institute of Medicine[114]. Phase 1 of the AMFm is being hosted and managed by the Roll Back Malaria Partnership, by invitation from the Global Fund. To achieve its stated goal, AMFm is expected to provide a payment to manufacturers of ACTs that meet recognized quality standards to reduce the price to first-line buyers (that is, governments, importers and wholesalers). This is intended to make these drugs more affordable to patients, even after mark-ups by distributors, taking costs to $0.50 or less for a full treatment course. This should not only increase the distribution of affordable ACTs throughout endemic regions but also increase their use compared with the less expensive but less effective CQ, SP and ART monotherapies (as well as the counterfeit ACTs). AMFm should also improve predictability in terms of supply demands, thus providing stability to the ACT production market. An important part of AMFm is in-country supporting interventions, designed to ensure that those suffering from malaria benefit from the reduced pricing. These not only include public education and awareness programmes but also provide training, pharmacovigilance and resistance monitoring programmes.

We conclude that at the very least, existing tools can reduce malaria morbidity and mortality worldwide to levels that have never been achieved before. However, this will require sustained financial support from implementation programmes and a detailed understanding of the pharmacological and genetic factors that affect antimalarial chemotherapy, specifically pharmacokinetic and pharmacodynamic properties, drug–drug interactions and mechanisms of resistance. If executed properly, these measures could yield an important achievement in global infectious diseases control and public health.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Glossary

Artemisinin-based combination therapy	A combination of artemisinin or one of its derivatives with one or more antimalarials of a different chemical class
Pharmacokinetic properties	Characteristics of a drug, including its mechanisms of absorption and distribution, the rate at which a drug action begins and the duration of the effect, the chemical changes of the agent in the body, and the effects and routes of excretion of drug metabolites
Antimalarial resistance	The ability of a parasite strain to survive and multiply despite the administration and adsorption of a drug given in doses equal to or higher than those usually recommended but within tolerance of the subject. The form of the drug that is active against the parasite must be able to gain access to the parasite or to the infected red blood cell for the duration that is necessary for its normal action
Recrudescence	The reappearance of asexual parasitaemia, after initial parasite clearance, that results from the same infection that caused the original illness
Pharmacodynamic properties	These include the physiological effects of a drug on the body, on microorganisms or on parasites in or on the body; the mechanisms of drug action; and the relationship between drug concentration and effect. Pharmacodynamics is often summarized as the study of what a drug does to the body; whereas pharmacokinetics is the study of what the body does to a drug
Gametocyte	A sexual form of the intra-erythrocytic *Plasmodium* parasite that matures over a 2-week period, after which it can transmit to *Anopheles* mosquito vectors. Following ingestion during the insect blood meal, a gametocyte transforms rapidly into a female or male gamete that can undergo sexual recombination in the mosquito midgut
Asexual blood-stage trophozoite	An asexual form of the intra-erythrocytic *Plasmodium* parasite that is undergoing cell growth and nuclear division, in preparation for parasite differentiation into a mature schizont that produces individual progeny (known as merozoites). These merozoites burst from the infected cell, ready to initiate new rounds of intracellular development
Selection pressure	Evolutionary pressure that allows certain genotypes to outcompete others. In the case of malaria, resistance to antimalarials disseminates owing to the selective survival advantage that resistant parasites have in the presence of the drug. In a given population, the greater the proportion of parasites that are exposed to antimalarials

	at concentrations that allow proliferation only of resistant parasites, the greater the selection pressure
Pharmacovigilance	The pharmacological science relating to the detection, assessment, understanding and prevention of adverse effects resulting from the short- or long-term use of medicines

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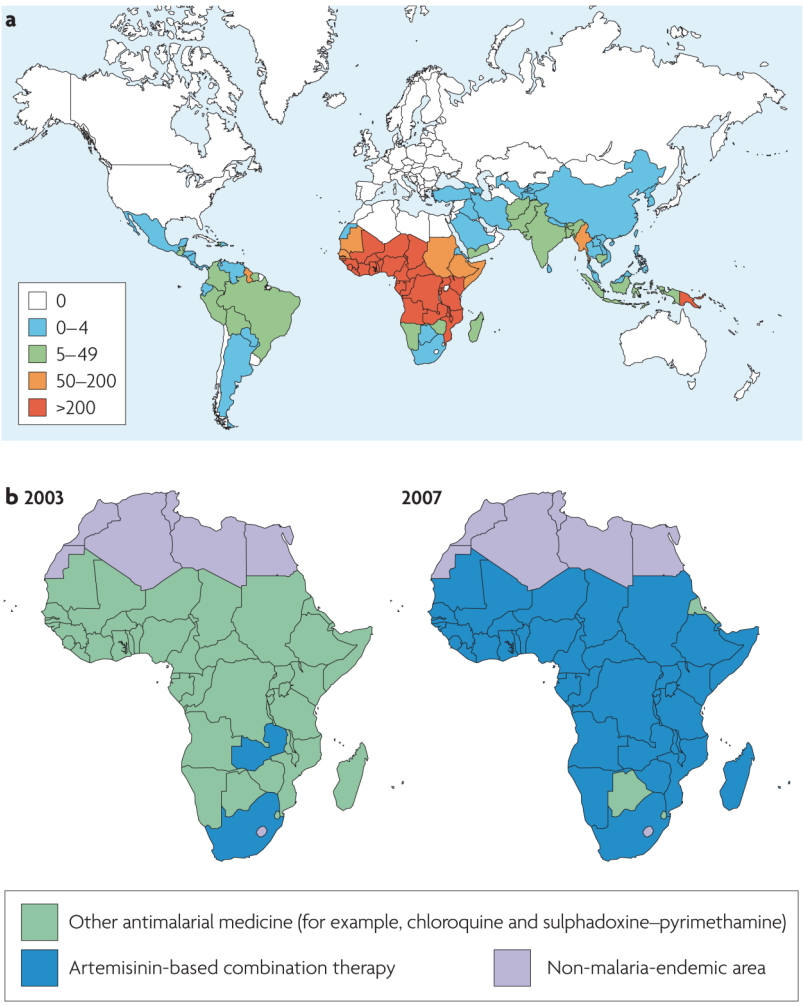

Figure 1. The worldwide incidence of malaria and the rapid adoption of artemisinin-based combination therapies across sub-Saharan Africa

a | The estimated incidence of malaria worldwide in 2006, stratified per 1, 000 population. Cases in Africa constituted 86% of the global total, Southeast Asia accounted for 9% and the eastern Mediterranean region had 3%. *Plasmodium falciparum* was found to be responsible for over 75% of the cases in most sub-Saharan African countries but was second to *Plasmodium vivax* in most countries outside Africa. **b** | The official first-line antimalarial policy in Africa in 2003 and 2007, demonstrating the dramatic shift from a diversity of first-line antimalarials (typically chloroquine or sulphadoxine–pyrimethamine) towards the adoption of artemisinin-based combination therapies. Part **a** image is modified, with permission, from REF. [106] © (2008) WHO. Part **b** image is modified, with permission, from REF. [140] © (2007) UNICEF.

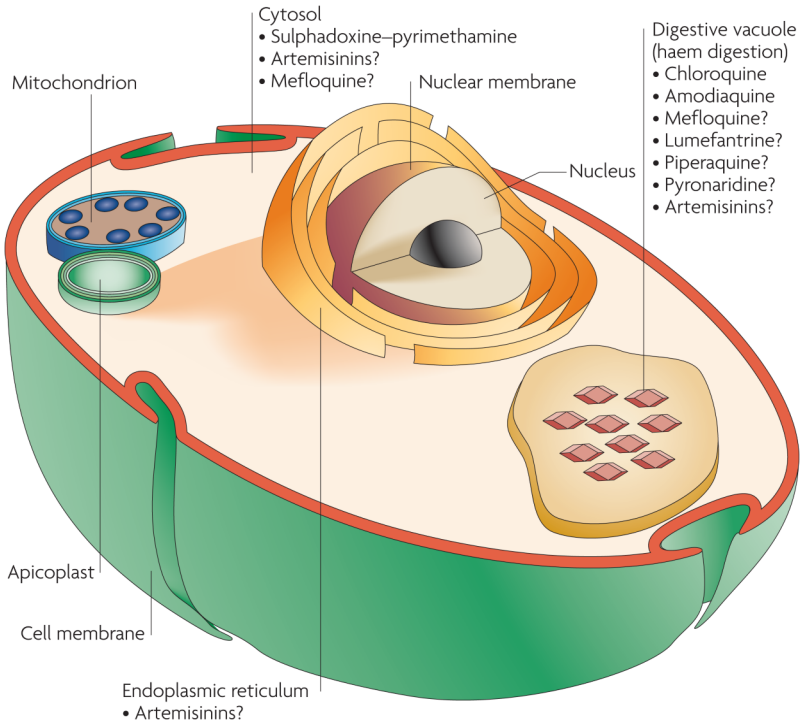

Figure 2. Site of action of antimalarial drugs

Depiction of an intra-erythrocytic *Plasmodium falciparum* parasite and the proposed target sites of several key antimalarials.

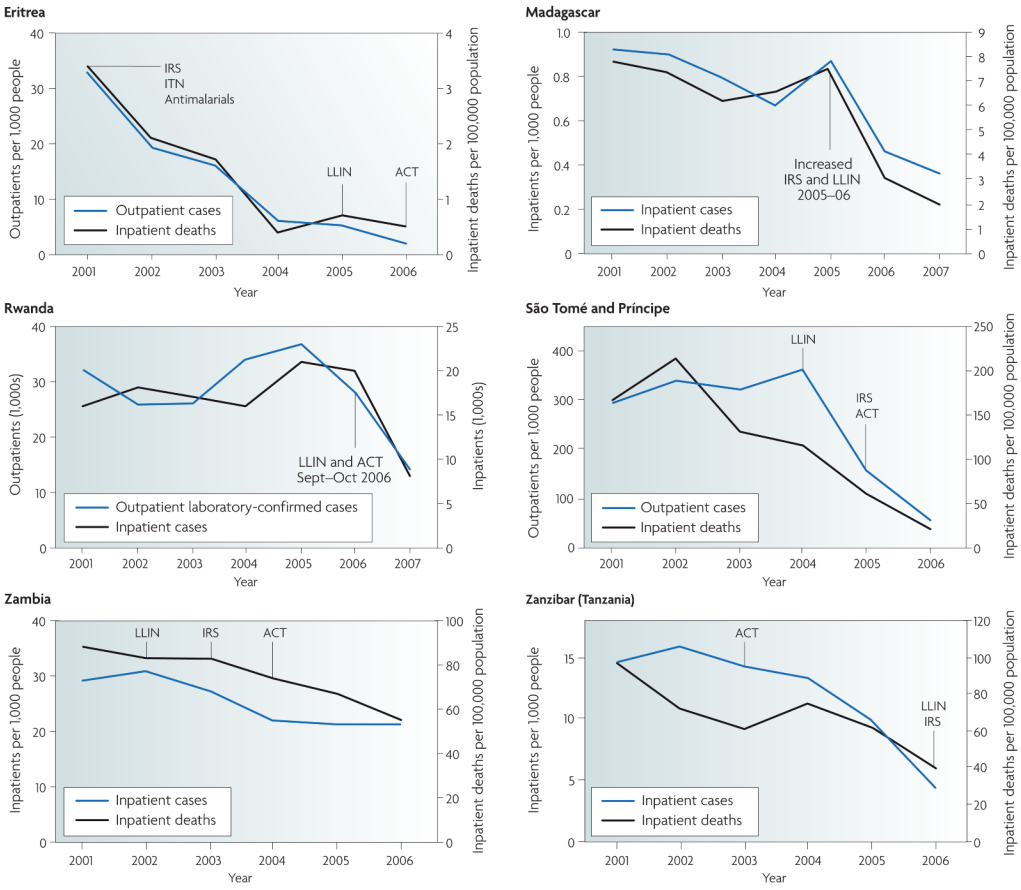

Figure 3. Recent trends in malaria cases and deaths

The graphs show the recent trends in malaria cases and deaths in six African countries following the implementation of malaria control programmes based on the use of: indoor residual spraying (IRS); insecticide-treated bed nets (ITN), including the more recent long-lasting insecticide-treated bed nets (LLIN); and an artemisinin-based combination therapy (ACT), typically artesunate–amodiaquine or artemether–lumefantrine. Figure is modified, with permission, from REF. [106] © (2008) WHO.

Table 1

Plasma half-lives of drugs used in artemisinin-based combination therapies

Antimalarial	$t_{1/2}$ of artemisinin derivative	$t_{1/2}$ of partner drug	Regions currently in use*
Artemether–lumefantrine	~3 hr	4–5 days	Africa, EM, SE Asia, WP and SA
Artesunate–mefloquine	<1 hr	14–21 days	Africa, SE Asia, WP and SA
Artesunate–amodiaquine	<1 hr	9–18 days‡	Africa and EM
Dihydroartemisinin–piperaquine	45 min	~5 weeks	SE Asia
Artesunate–pyronaridine§	<1 hr	16 days	NA
Chloroquine‖	NA	1–2 months	Africa, EM, SE Asia, WP and SA
Sulphadoxine–pyrimethamine‖	NA	~4 days (S) or ~8 days (P)	Africa, EM (IPT in Africa, EM and WP)

*Data from REFS 106,140.

‡This refers to the $t_{1/2}$ of the active metabolite monodesethylamodiaquine; the $t_{1/2}$ of amodiaquine is ~3hr.

§Recently completed Phase III trials.

‖These former first-line antimalarials are included as a reference. EM, eastern Mediterranean; IPT, intermittent preventive treatment; NA, not applicable; P, pyrimethamine; S, sulphadoxine; SA, South America; SE Asia, Southeast Asia; $t_{1/2}$, half-life; WP, Western Pacific.