FEBS Letters 585 (2011) 1551–1562

journal homepage: www.FEBSLetters.org

Review

# Drug-resistant malaria: Molecular mechanisms and implications for public health

Ines Petersen [a,*], Richard Eastman [b], Michael Lanzer [a,*]

[a] Department of Infectious Diseases, Parasitology, Universitätsklinikum Heidelberg, Im Neuenheimer Feld 324, 69120 Heidelberg, Germany
[b] Malaria Functional Genomics Section, Laboratory of Malaria and Vector Research, NIAID, DIR, NIH, Rockville, MD 20852, USA

ARTICLE INFO

*Article history:*
Received 29 March 2011
Revised 15 April 2011
Accepted 18 April 2011
Available online 23 April 2011

Edited by Sergio Papa, Gianfranco Gilardi and Wilhelm Just

*Keywords:*
Drug resistance
Antimalarial drug
*pfcrt*
*pfmdr1*
*Plasmodium falciparum*
*Plasmodium vivax*

ABSTRACT

**Resistance to antimalarial drugs has often threatened malaria elimination efforts and historically has led to the short-term resurgence of malaria incidences and deaths. With concentrated malaria eradication efforts currently underway, monitoring drug resistance in clinical settings complemented by in vitro drug susceptibility assays and analysis of resistance markers, becomes critical to the implementation of an effective antimalarial drug policy. Understanding of the factors, which lead to the development and spread of drug resistance, is necessary to design optimal prevention and treatment strategies. This review attempts to summarize the unique factors presented by malarial parasites that lead to the emergence and spread of drug resistance, and gives an overview of known resistance mechanisms to currently used antimalarial drugs.**


## 1. The burden of drug resistance in malaria

Malaria, together with tuberculosis and HIV, is an important cause of morbidity and mortality, especially among children [1,2]. The disease is caused by the protozoan parasite *Plasmodium*, and is transmitted by an Anopheline mosquito vector [3]. The five *Plasmodia* species affecting humans are *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale*, *Plasmodium malariae*, and *Plasmodium knowlesi*, together causing approximately 225 million incident infections per year, resulting in nearly one million deaths [1,4]. Among them, *P. falciparum* is the most prevalent malaria species worldwide, especially in Africa, causing the most severe form of the disease and being responsible for over 90% of the deaths. *P. vivax* is the second most common species, located mainly in Asia and South America, and can cause a relapsing form of malaria [3,5].

The battle against malaria started with the discovery by Ross and Grassi in 1898, showing that the transmission of malaria parasites occurs through the bite of an infected mosquito [6]. This finding formed the basis of initial malaria control measures, including the installation of window and door screens and reduction of mosquito breeding sites through changes in agricultural habits and the application of insecticides, namely dichloro-diphenyl-trichloroethane (DDT). These interventions functioned by limiting disease transmission, and eliminated the disease from more than 10 countries between 1900 and 1946 [6]. In 1955, the World Health Organization launched the "Global Malaria Eradication Programme" and chloroquine chemotherapy was implemented to complement the initial vector control measures. When the program was officially ended in 1969, an additional 27 countries were declared malaria-free [6]. Unfortunately, elimination of malaria could not be achieved in most underdeveloped countries (sub-Saharan Africa was omitted from the original eradication program), resulting in the current predominant distribution of malaria to sub-tropical and tropical regions [1] (Fig. 1A). Among the reasons for the eventual halt to the eradication effort were widespread resistance to available insecticides, wars and massive population movements, difficulties in obtaining sustained funding from donor countries, the lack of community participation, and finally, the emergence of chloroquine resistant malaria in Southeast Asia and South America around 1960 [7]. The subsequent spread of chloroquine resistant *P. falciparum* to Africa and lack of an effective, affordable alternative ultimately led to a 2- to 3-fold increase in malaria-related deaths in the 1980s [8]. The only viable alternative to chloroquine, at that time, was sulfadoxine–pyrimethamine, however, it also encountered drug-resistant parasites about a year after implementation [9]. Several other antimalarial drugs have since been deployed to combat parasites resistant to chloroquine and sulfadoxine–pyrimethamine, including mefloquine, amodiaquine and quinine.

* Corresponding authors. Fax: +49 6221 564643 (M. Lanzer).
*E-mail addresses:* i.petersen@uni-heidelberg.de (I. Petersen), michael_lanzer@med.uni-heidelberg.de (M. Lanzer).

0014-5793/$36.00 
doi:10.1016/j.febslet.2011.04.042

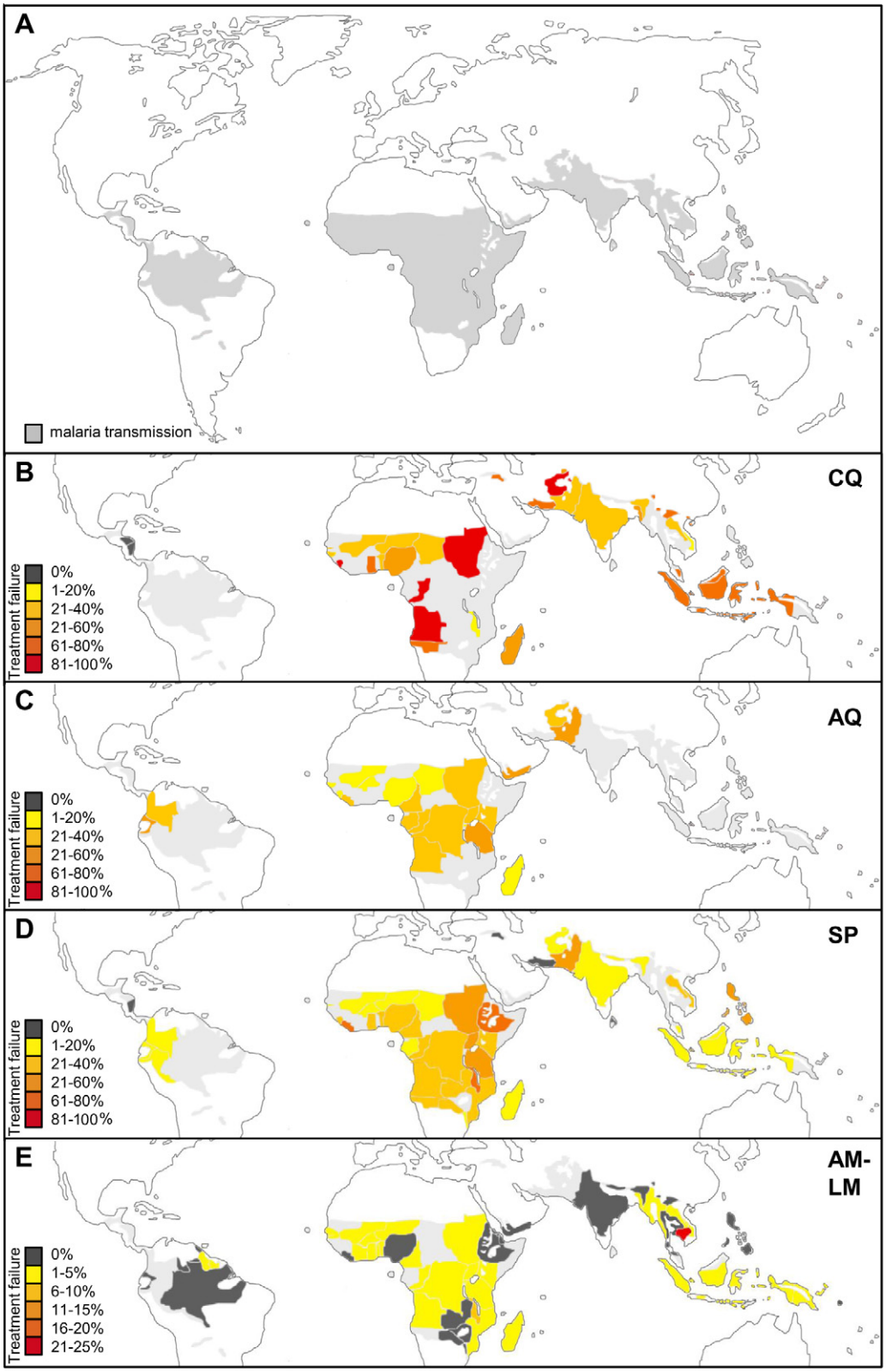



**Fig. 1.** Efficacy of selected antimalarial drugs and combination therapies in areas with malaria transmission. World map adapted from the World Health Organization "Malaria, countries or areas at risk of transmission, 2009" [147]. (A) Endemicity map showing the areas and countries where malaria transmission occurred in 2009. (B–E) The graphic representation of the median percentages of treatment failure data collected from clinical trials with a minimum of 28 day of follow-up, published in the "Global Report on Antimalarial Drug Efficacy and Drug Resistance: 2000–2010" by the WHO [148]. (B) Chloroquine treatment failure; (C) amodiaquine treatment failure; (D) sulfadoxine–pyrimethamine (Fansidar) treatment failure; (E) artemether–lumefantrine (Coartem) treatment failure. Please note that the scale in (E) differs from (A–D). *Abbreviations:* CQ: chloroquine; AQ: amodiaquine; SP: sulfadoxine–pyrimethamine; AM: artemether (an artemisinin derivative); LM: lumefantrine.

The historic usage of these replacement drugs in monotherapy has now similarly resulted in the selection of resistant parasites, at least in some parts of the world. In 1998, another attempt to roll back malaria was launched and has been relatively successful, with a reduction in malaria-related mortality by about 20% from 985 000 in 2000 to 781 000 in 2009 [1]. The main pillars of the current efforts have been vector control, including long-lasting insecticide treated bed nets and indoor residual insecticide spraying (the spraying of insecticides onto walls within dwellings), along with improved diagnostics and usage of effective chemotherapy to treat infected individuals, thereby curing the infection and reducing further transmission [1]. Currently, the most effective treatment for malaria are artemisinin-based combination therapies (ACTs) that combine a semi-synthetic derivative of artemisinin, a chemical compound isolated from the plant *Artemisia annua*, with a partner drug of a distinct chemical class. ACTs compensate for the poor pharmacokinetic properties of the artemisinins, increase treatment efficacy, and are thought to reduce the emergence of drug-resistant parasites [10].

Unfortunately, recent alarming reports observed the emergence of artemisinin resistant parasites in Southeast Asia, which could derail the current elimination/eradication efforts, and again foster an increase in malaria cases and deaths [11,12]. The emergence and spread of drug resistance does not only lead to an increase in treatment failures and mortality, but also augments the costs associated with treatment and control efforts on the level of both the affected individual (resulting from treatment, purchase of bed nets and absenteeism from work) and the government (for vector control, health facilities, education and research) [13].

In summary, the emergence of drug-resistant parasites severely impairs control efforts and has to be contained or circumvented with the use of alternative treatments. For this, a deeper knowledge of regional drug resistance patterns, understanding of the mechanisms of action of the currently used drugs, appreciation of cross-resistance between drugs and elucidation of genetic markers for the surveillance of resistance are essential to rationally design an individualized effective drug policy in all malaria-affected countries.

## 2. Emergence and spread of drug resistance in malaria parasites

The emergence of resistance in *Plasmodium* depends on multiple factors, including (i) the mutation rate of the parasite, (ii) the fitness costs associated with the resistance mutations, (iii) the overall parasite load, (iv) the strength of drug selection, and (v) the treatment compliance.

The mutation rate of the parasite has a direct influence on the frequency at which resistance can emerge. While higher mutation rates enable a faster emergence of resistance they can also lead to an accumulation of deleterious mutations. In *P. falciparum* the mutation rate was determined to be approximately $10^{-9}$ from experiments measuring spontaneous mutations in the *pfdhfr* gene, which is relatively low [14]. An increased mutation rate is advantageous for the adaptation to quickly changing environments [15]. This is exactly the situation that parasites are exposed to upon changing drug selection pressures. Some studies describe an "accelerated resistance to multiple drugs" (ARMD) phenotype, present in isolates from Southeast Asia, which acquired drug resistance at a higher rate than other geographically distinct strains in in vitro experiments [16]. Such ARMD parasites could explain the observation that resistance to new drugs often arises first in Southeast Asia [16].

Since mutations associated with drug resistance often impart a fitness cost, the selective advantage acquired by becoming drug-resistant is balanced by the biological cost arising from the altered function of the mutated protein. Such a fitness cost can be mitigated by the acquisition of compensatory mutations during prolonged drug pressure [17].

Additionally, it has been postulated that high parasitemias (*P. falciparum* infections rise to $10^{10}$–$10^{12}$ parasites within an individual) can lead to faster elimination of deleterious mutations and enhance selection of compensatory mutations [18].

The emergence of drug-resistant parasites can also be accelerated by strong drug selection pressure, which decreases the prevalence of competing sensitive wild type parasites.

Inadequate drug exposure through improper dosing, poor pharmacokinetic properties, fake drugs, or infections acquired during the drug elimination phase of a prior antimalarial treatment can all result in parasites exposed to sub-optimal drug concentrations, which increases the probability for drug-resistant parasites to arise [19]. In high-transmission regions, infections acquired after treatment from a previous malaria episode are common and result in the exposure of parasites to sub-therapeutic drug concentrations. This, in turn, selects for drug-tolerant parasites, which may represent an intermediate stage to full resistance [20]. The time during which sub-therapeutic concentrations are present within the patient is prolonged in antimalarials that possess a long half-life, a drawback that is often balanced with the provided beneficial prophylactic effect this also imparts [21]. To mitigate the length of time that drugs exist in sub-optimal concentration, treatment guidelines should be strictly adhered to. However, this is a particular challenge in areas of high migration across borders, such as along the Thailand–Burma border, and in areas of political or social unrest, which disrupts prompt access to medical care and public health measures, perpetuating the emergence and spread of resistant parasites [22].

Transmission is another critical step for the spread of drug-resistant parasites. The intensity of transmission has an important role in determining if parasites are effectively transmitted during a mosquito blood meal. Drug-resistant parasites emerging in a high-transmission area would likely be present in a polyclonal infection (up to 7 clones have been reported to coexist within one host [23]). If mutations conferring drug resistance are associated with a significant fitness cost, they are more likely to be outcompeted by sensitive parasites and not transmitted efficiently. The spread of resistant parasites is also affected by the impact of antimalarials on the gametocytes, which are the transmissible stages of the parasite. Artemisinins have been shown to decrease the number of gametocytes carried by a patient, thereby reducing transmission [24]. In contrast, both sulfadoxine–pyrimethamine and chloroquine elevate the gametocytemia [25]. In addition, drug resistance may enhance transmission if drug selection pressure diminishes the viability of sensitive gametocytes in a polyclonal infection, increasing the propensity for transmitting drug-resistant parasites [26].

## 3. Drugs used against malaria

Important attributes for the successful implementation of antimalarial drugs are good tolerability and safety (especially in young children), affordability, availability in endemic countries and short course regimens. Primarily to decrease the emergence of drug-resistant parasites, almost all antimalarials are now to be administered as part of a combination therapy, with each drug targeting distinct mechanisms within the parasite.

### 3.1. Quinine

Quinine, an aryl-amino alcohol, is one of the oldest antimalarial agents and has been used by the native population of Peru for

centuries in the form of pulverized bark of the cinchona tree to treat fevers and chills. In 1820, the active alkaloid from the bark was isolated and named quinine [27]. Synthesis, first described in 1944, was complicated and availability issues during the world wars prompted scientists to develop synthetic alternatives to quinine, among them chloroquine, which eventually replaced quinine for routine treatment [27]. Quinine is now used to treat severe cases of malaria and, as a second line treatment, in combination with antibiotics to treat resistant malaria. Its short half-life of 8–10 h likely contributed to the scarcity of widespread quinine resistance; however, several reports have indicated the emergence of quinine resistance in vivo [28,29]. The molecular mechanism by which quinine acts against *P. falciparum* is only partially understood. Similar to chloroquine, quinine has been demonstrated to accumulate in the parasite's digestive vacuole (DV) and can inhibit the detoxification of heme, an essential process within the parasite [30]. Recent studies show that the genetic basis for resistance to quinine is complex, with multiple genes influencing susceptibility. Currently, three genes have been associated with altered quinine response: *pfcrt* (*P. falciparum chloroquine resistance transporter*), *pfmdr1* (*P. falciparum multidrug resistance transporter 1*), and *pfnhe1* (*P. falciparum sodium/proton exchanger 1*), all of them encoding for transporter proteins [31–34].

### 3.2. Chloroquine

Chloroquine is a 4-aminoquinoline that was introduced in the late 1940s and used on a massive scale for malaria treatment and prevention. Its efficacy, affordability and safety, even during pregnancy, made it the gold standard treatment of malaria for many years [35]. Chloroquine has one of the longest half-lives among antimalarials with approximately 60 days, which provides a chemoprophylactic effect during the drug elimination phase but also exposes the parasites to an extended time period after which chloroquine has fallen below the therapeutic concentration, which may select for drug-resistant parasites [20]. Chloroquine resistant parasites emerged approximately 10 years after its introduction, first along the Thai–Cambodian border and also in Colombia in the late 1950s [36]. Genetic epidemiological data suggests that resistance then spread from Southeast Asia to Africa in the late 1970s. Additionally, resistance also emerged independently from other foci, including Papua New Guinea and the Philippines [36]. Chloroquine resistant *P. falciparum* is now predominant in nearly all malaria endemic regions (Fig. 1B), but despite widespread resistance, chloroquine maintains some clinical efficacy in areas where patients have acquired partial immunity to malaria (premunition), through repeated infections. This indicates that even against resistant parasites chloroquine does maintain some efficacy, although not enough to solely clear the infection [37]. Chloroquine is also the first-line treatment of *P. vivax* infections, however, the prevalence of chloroquine resistant *P. vivax* is increasing [1].

Chloroquine's mechanism of action has been an intense area of research for decades and evidence supports that the principal target is the heme detoxification pathway in the DV, where the parasite degrades erythrocytic hemoglobin and polymerizes the liberated toxic heme monomers to inert biocrystals of hemozoin [38]. Chloroquine is a weak base with p$K_a$ values of 8.1 and 10.2 and therefore a proportion of the drug remains uncharged at the neutral pH of the blood [39]. This allows chloroquine to diffuse freely across membranes. However, when chloroquine encounters the acidic DV, it becomes diprotonated and unable to transverse across the membrane [39]. As it accumulates in the DV, chloroquine binds to hematin, a heme dimer [30]. This interaction prevents the detoxification of free heme, leading to the buildup of heme monomers that permeabilize the membrane, resulting in the eventual death of the parasite [40]. Polymorphisms in PfCRT have been demonstrated to be the main chloroquine resistance determinant [41]. In some parasite strains PfMDR1 can also modulate the degree of chloroquine resistance [42], indicating that some alleles and overexpression of PfMDR1 may increase the concentration of chloroquine within the DV by active transport (Fig. 2A). Interestingly, studies have demonstrated linkage disequilibrium between PfMDR1 and PfCRT alleles in chloroquine resistant parasites in Southeast Asia and Africa, suggesting a functional interaction of both proteins [26,43].

### 3.3. Amodiaquine

Amodiaquine, also a 4-aminoquinoline, is structurally related to chloroquine and has been in use for more than 70 years [20,44]. Amodiaquine has a short half-life of 3 h, thus the antimalarial activity is thought to be exerted by the primary metabolite, monodesethylamodiaquine, which has a half-life of 9–18 days [20]. Based on structural similarity, amodiaquine is hypothesized to act by inhibiting heme detoxification, and has been shown to accumulate within the DV and to bind to heme in vitro [45,46]. Cross-resistance between chloroquine and amodiaquine has been reported and mutations in PfCRT and PfMDR1 are associated with decreased susceptibility to both drugs. However, cross-resistance is incomplete and some chloroquine resistant parasites remain susceptible to amodiaquine (see below) (Figs. 1C and 2A) [44].

### 3.4. Mefloquine

Mefloquine is a 4-methanolquinoline with a long half-life of 14–18 days [20], which was first introduced in the 1970s [47]. Resistance to mefloquine is mediated by amplification of *pfmdr1*, leading to overexpression of this resident DV membrane transporter [48]. Although the exact mechanism of action remains unclear, in vitro experiments demonstrate that mefloquine can bind to heme and exert some antimalarial activity by inhibiting heme detoxification [49,50]. However, studies on transgenic parasites expressing different *pfmdr1* copy numbers, observed a reduced parasite susceptibility to mefloquine with increased PfMDR1-mediated import into the DV [51,52], suggesting a primary mode of action outside of the DV (Fig. 1B) [46]. Additionally, it has been shown that mefloquine inhibits the import of other solutes into the DV and might therefore also target the PfMDR1 transport function itself [51].

### 3.5. Piperaquine

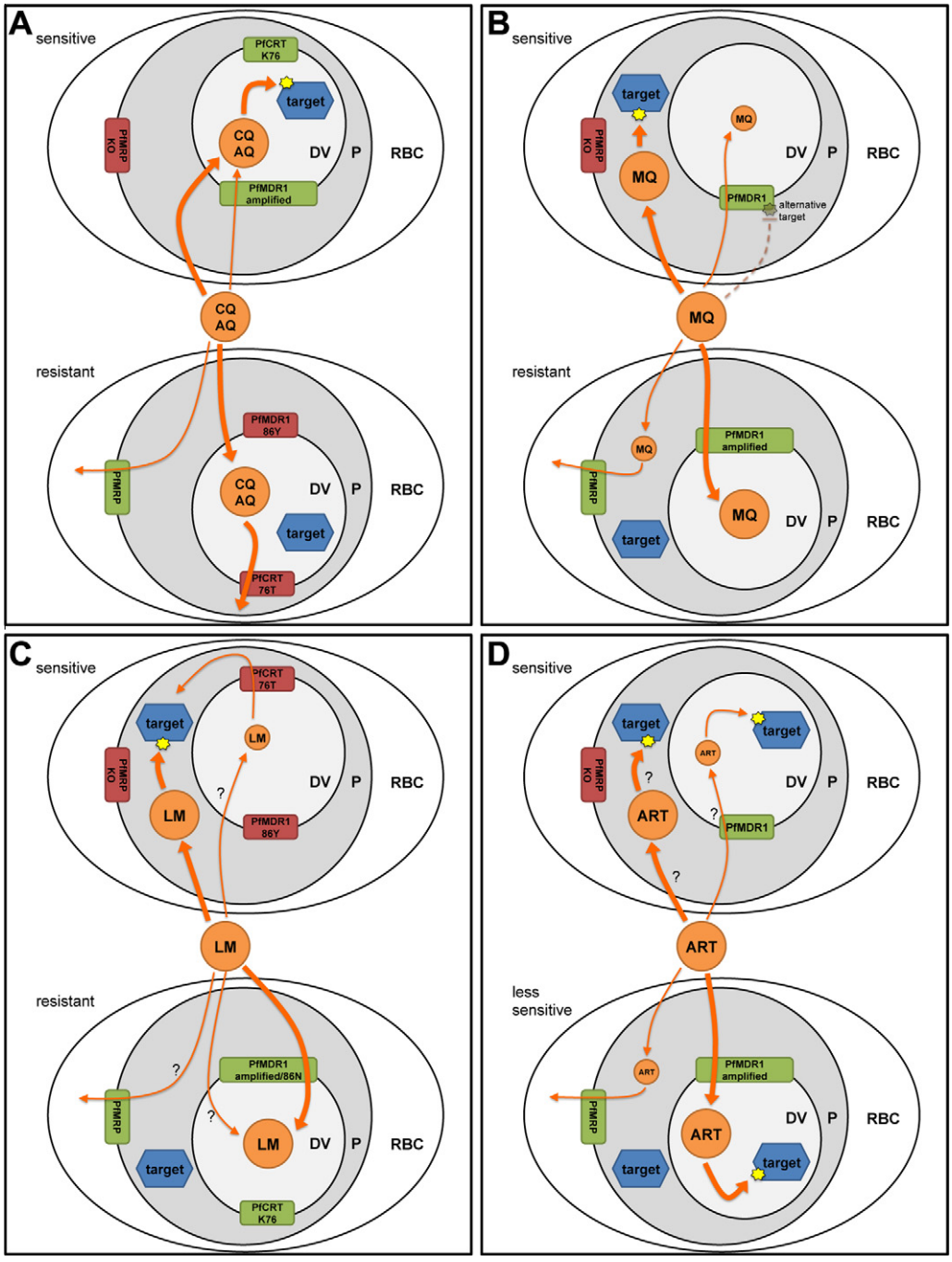
The bis-4-aminoquinoline piperaquine possesses an extended half-life of approximately 5 weeks. Due to structure similarities with chloroquine, it has been postulated that piperaquine has a similar mode of action to chloroquine. Although the exact mechanism of action is unclear, studies have shown that piperaquine accumulates in the DV and that it is a potent inhibitor of heme polymerization [53,54]. In addition, electron microscopic studies using a mouse malaria model, have revealed morphologic changes of the DV and clumping of hemozoin in trophozoites after exposure to piperaquine, further implicating the DV as a site of action [55,56]. Intensive piperaquine monotherapy in China during the late 1970s has led to the emergence of resistant *P. falciparum* strains, resulting in the subsequent decline in its use. Piperaquine was later "rediscovered" and is now employed as a partner drug in an artemisinin-based combination therapy [56]. Modulation of piperaquine susceptibility by mutations in PfCRT have been confirmed, however, the shift in piperaquine response was modest [57], and the clinical relevance of this finding remains unclear.

### 3.6. Lumefantrine

Lumefantrine (previously known as benflumetol) is structurally related to the hydrophobic arylamino alcohol antimalarials. The half-life of lumefantrine is about 3–5 days and absorption of this lipophilic drug can vary between individuals [58], requiring the co-ingestion with a high-fat meal to increase the oral bioavailability [59]. Polymorphisms in PfMDR1, particularly the variant N86, and amplification of the encoding gene (*pfmdr1*) have been associated with reduced susceptibility to lumefantrine in Africa and Asia [60–62]. Additionally, parasites with the wild type copy of PfCRT show reduced susceptibility to lumefantrine, as indicated by both field studies and in vitro assays [63]. The inverse correlation between lumefantrine and chloroquine susceptibilities is quite interesting and may prove useful in regards to combination therapies (Fig. 2C) [63].

### 3.7. Primaquine

Primaquine is an 8-aminoquinoline with a half-life of approximately 6 h [64] and is currently the only approved therapy for the treatment of *P. vivax* hypnozoite liver stages [65]. Unfortunately, primaquine is contraindicated in patients with certain subclasses of glucose-6-phosphate dehydrogenase (G6PD, encoded on the X chromosome) deficiency, due to the risk of a severe reaction resulting in hemolytic anemia. Prevalence of G6PD deficiency varies in males in different endemic regions, with 0.9–28.1% in Africa, 0.7–10.8% in Southeast Asia, 6.1–29% in the Middle East and <2% in



South America [66]. Several studies have suggested that primaquine binds to PfCRT and can thereby inhibit chloroquine transport, possibly leading to a synergistic action between the two antimalarials and a reversal of chloroquine resistance [67–69].

### 3.8. Atovaquone

Atovaquone is a lipophilic hydroxynaphthoquinone analog structurally related to ubiquinol (an important coenzyme in the electron transport chain within the mitochondria) and is used for treatment of apicomplexan parasites, including *Plasmodium*, *Toxoplasma*, *Theileria* and *Babesia* [70]. It has a blood plasma half-life of 2–3 days [71,72]. Molecular evidence exists that atovaquone specifically targets the cytochrome $bc_1$ complex, located in the inner mitochondrial membrane, thereby inhibiting the respiratory chain. In *P. falciparum* blood stage parasites the respiratory chain is required for the regeneration of ubiquinone, the electron acceptor for dihydroorotate dehydrogenase, which is an essential enzyme for pyrimidine biosynthesis [73]. Atovaquone is currently used in combination with proguanil (Malarone), mainly as a prophylactic medication for tourists, due to the high costs and easily arising resistance, which is conferred by single nucleotide polymorphisms in the cytochrome *b* gene [74].

### 3.9. Antifolate drugs

The antifolate drugs used for malaria therapy are the sulfa drugs sulfadoxine and dapsone that inhibit the dihydropteroate synthetase enzyme (PfDHPS), and pyrimethamine and proguanil, which inhibit the dihydrofolate reductase (PfDHFR) activity of the bifunctional dihydrofolate reductase/thymidylate synthase enzyme. In addition to PfDHFR, proguanil may target other pathways [75]. The drug combination sulfadoxine–pyrimethamine (Fansidar), which possesses a half-life of approximately 4–5 days, was introduced in the 1970s after the emergence of parasites resistant to chloroquine. Sulfadoxine–pyrimethamine was a highly effective, cheap, well-tolerated drug combination with good compliance rates due to being administered in a single dose. Unfortunately, resistance due to point mutations in both target enzymes emerged quickly after introduction (Fig. 1D) [76]. Therefore, sulfadoxine–pyrimethamine is now primarily used as intermittent preventative malaria treatment during pregnancy and, to a lesser extent (due to high prevalence of parasites resistant to the drug combination in endemic regions), for the treatment of malaria infection. The two other antifolate drugs, dapsone and proguanil, were also administered as a combination, Lapdap. Unfortunately dapsone caused hemolysis in G6PD deficient patients, and therefore this combination therapy is no longer recommended [77].

### 3.10. Artemisinins

Since the emergence of resistance to almost all quinolone and antifolate drugs in *P. falciparum* malaria, artemisinin and its derivatives have been used as a replacement and now constitute the linchpin in antimalarial chemotherapy worldwide. Artemisinin is a natural product, isolated from the *A. annua* plant (Chinese wormwood) and has been used in China as an herbal remedy for many centuries. Artemisinins have a unique trioxane structure with an endoperoxide bond that is required for antimalarial activity [50]. Due to the low solubility of artemisinin, several semi-synthetic derivatives are used clinically (artemether, artesunate, and dihydroartemisinin) [78]. Among the current antimalarials, artemisinins have the shortest half-lives of 0.5–1.4 h [79]. Initial widespread use of artemisinin derivatives as monotherapy in Southeast Asia has likely contributed to the reduced efficacy observed in recent years along the Thai–Cambodian border, which has been characterized as a delay in the parasite clearance time (Fig. 1E) [11,50]. The mechanism of action of artemisinin drugs is not fully understood, but the prevailing theory is that the endoperoxide bridge is cleaved, leading to the formation of reactive carbon radicals that subsequently alkylate essential biomolecules [78].

Mutations in *pfatp6* (*P. falciparum* $Ca^{2+}$ transporting ATPase 6) have been associated with decreased artemether susceptibility in field isolates from French Guyana [80] and polymorphisms in

**Fig. 2.** Hypothetical models for antimalarial drug transport pathways and drug target localization. The three transporter proteins PfCRT, PfMDR1 and PfMRP are major determinants in resistance to several important antimalarial drugs. PfCRT and PfMDR1 reside within the DV membrane [89,110], demonstrating the importance of this acidic organelle in modulation of drug susceptibility, possibly either by serving as a compartment in which toxic drugs are sequestered away from their targets outside of the DV, or as a compartment, which harbors the target itself, and where the accumulation is limited by the transporters. PfMRP, located at the parasitic plasma membrane, possibly acts as a general drug efflux pump [121]. Parasite susceptibility states, influenced by variants of the three transporters (PfMRP/PfMRP-KO, PfMDR1/PfMDR1-86N/PfMDR1-Y86/PfMDR1-amplified, PfCRT-K76/PfCRT-76T) to five antimalarial drugs (chloroquine, amodiaquine, mefloquine, lumefantrine, and artemisinins) and the target localization, deducted from the suggested drug accumulation sites, are depicted. (A) Chloroquine and amodiaquine are structurally similar 4-aminoquinoline drugs and parasites generally demonstrate cross-resistance between them [149]. Therefore, a similar transport pathway and drug target for both drugs is suggested. The majority of drug accumulates within the DV through passive diffusion, but may be increased through the activity of PfMDR1, since susceptibility is increased in parasites overexpressing PfMDR1 [42]. Parasites obtain resistance to both drugs when PfCRT 76T present, which effluxes both antimalarials out of the DV, the location of the drug target [102]. However, there are small variations in the strength of amodiaquine resistance depending on additional mutations in PfCRT (mainly in amino acids 72–75) while this is not the case for chloroquine [44]. The N86Y polymorphisms in PfMDR1 can further increase resistance [150], putatively decreasing the active transport into the DV. Additionally, PfMRP presumably aids active efflux, at least for chloroquine [121]. (B) The primary determinant conferring resistance to mefloquine is amplification of *pfmdr1*, resulting in increased expression [42,48]. One mechanism of action for mefloquine resistance therefore might be that overexpression of PfMDR1 leads to accumulation of mefloquine in the DV and sequestration of the drug away from its hypothetical drug target located outside of the DV. Alternatively, the observation that mefloquine inhibits PfMDR1 transport activity [51] also could lead to the hypothesis that mefloquine directly targets PfMDR1 function. PfMRP acts as a general drug efflux pump, reducing the concentration of mefloquine within the parasite. (C) There exist little data on the mechanistic target of lumefantrine. However, current evidence demonstrates an inverse cross-resistance with chloroquine and an influence of PfCRT and PfMDR1 haplotype and PfMDR1 copy number on lumefantrine susceptibility [63]. Wild type PfMDR1 haplotype (N86), and *pfmdr1* amplification both can lead to lumefantrine resistance [52,61]. Additionally, it has been shown that wild type PfCRT (K76) also decreases susceptibility [63]. If drug transport follows similar patterns as suggested for chloroquine, amodiaquine and mefloquine, lumefantrine accumulates in the DV through active PfMDR1 transport in lumefantrine resistant parasites and potentially additional transport pathways. Parasites that possess a mutated form of PfCRT (76T) may display increased susceptibility by the active transport of this mutated protein out of the DV. This suggests that the primary target of lumefantrine is found in the cytosol or another parasite organelle. Although untested, PfMRP may be capable of actively transporting lumefantrine. (D) The drug targets of artemisinin and its derivatives remain elusive, but it was suggested that artemisinin is activated by ferrous iron and subsequently alkylates molecules such as heme and specific proteins, thereby disturbing the parasite's metabolism and ultimately causing its death [50]. Ferrous iron is present both in the DV as well as outside of the DV [151]. Therefore, artemisinin could be activated and alkylate heme and proteins in both compartments. PfMDR1 amplification leads to a weak but significant decrease of artemisinin susceptibility, suggesting an accumulation of artemisinin in the DV [83]. Additionally, a knock-out of PfMRP increases artemisinin susceptibility [121], indicating that it acts as an artemisinin efflux transporter. From the current data, higher concentrations in the cytosolic compartment correlate with increased sensitivity, suggesting that inactivation of cytosolic enzymes and proteins is more detrimental to the parasite's survival than its action within the DV. *Abbreviations:* PfCRT: *P. falciparum* chloroquine resistance transporter; PfMDR1: *P. falciparum* multidrug resistance transporter 1; PfMRP: *P. falciparum* multidrug resistance protein; CQ: chloroquine; AQ: amodiaquine; MQ: mefloquine; LM: lumefantrine; ART: artemisinin (and derivatives); KO: knock-out; RBC: red blood cell; P: parasite; DV: digestive vacuole.

*ubp1*, encoding for a deubiquitination enzyme, are associated with increased artesunate resistance in the rodent malaria parasite *Plasmodium chabaudi* [81]. However, these two candidates do not show consistent association with parasites demonstrating an elongated parasite clearance time, the currently described in vivo phenotype of *P. falciparum* parasites with reduced artemisinin susceptibility [82]. Although not mediating complete resistance to artemisinin derivatives, *pfmdr1* amplification can significantly reduce parasite susceptibility [52,82,83]. Additionally, some of the observed clinical phenotypes may be attributed to parasites entering a quiescent state during the ring stage of intraerythrocytic development when exposed to artemisinin derivatives, thereby avoiding drug selection pressure [84]. Whatever mechanism is mediating the observed clinical phenotype, recent genetic analysis has demonstrated that the reduction in artemisinin susceptibility has a heritable component [12].

To prolong the lifespan of artemisinin drugs by reducing the emergence of drug-resistant parasites, artemisinin derivatives are administered in combination with at least one other antimalarial compound [85]. Current artemisinin-based combination therapies include artemether–lumefantrine (Coartem, presently the most commonly used ACT worldwide), artesunate–mefloquine, artesunate–amodiaquine, artesunate–sulfadoxine–pyrimethamine, dihydroartemisinin–piperaquine, and artesunate–pyronaridine [1].

## 4. Drug resistance genes and resistance mechanisms

Efficient drug treatment involves the targeting of an essential biological process, which differs from or is non-existent in the host. *Plasmodium* is highly adapted to its unique living environments and many genes show little or no sequence similarity to other genes encoding characterized proteins [86]. This creates a number of putative drug targets; however, with little information to evaluate the functionality or essential nature of these determinants, target validation becomes a challenge. Additionally, the field is hampered by scarce or inefficient genetic tools for the analysis and identification of candidate genes in this haploid organism [87].

Linkage studies of three available genetic crosses, investigation of field isolates and examination of candidate genes have led to the identification of the following genes responsible for resistance to the most important antimalarial drugs. These have been implemented as molecular markers to screen for the emergence of resistance and assess its spread, as a means to inform rational drug policy decisions.

### 4.1. PfCRT

Intensive chloroquine chemotherapy has led to the emergence of resistant *P. falciparum* strains. Analysis of the progeny from a genetic cross between the chloroquine sensitive strain HB3 from Honduras and Dd2, a chloroquine resistant strain from Indochina, identified a 36 kb region on chromosome 7 responsible for chloroquine resistance [88]. Subsequent examination of the locus identified the *P. falciparum* chloroquine resistance transporter gene (*pfcrt*), encoding a transmembrane protein of 424 amino acids and 48.6 kDa localized within the DV membrane (MAL7P1.27) [89]. Thus far, no crystal structure is available and little is known about the tertiary structure or native function of PfCRT. Bioinformatic analysis has predicted PfCRT to be a member of the drug/metabolite transporter family with 10 transmembrane domains and both N- and C-termini facing the cytosolic side of the organelle [90]. Homologs of PfCRT have been identified in several *Plasmodia* species (*P. vivax*, *Plasmodium yoelii*, *P. chabaudi*, *P. knowlesi*, *Plasmodium berghei*) and CRT-like proteins exist in non-related organisms such as *Cryptosporidium parvum*, *Dictyostelium discoideum*, and *Arabidopsis thaliana* [90]. Attempts to genetically disrupt *pfcrt* have failed [91], suggesting an essential function in the parasite. Underlining the important function of this transporter, mutant PfCRT haplotypes in Africa and Asia have been shown to confer a fitness cost to the parasite, although the strength of the fitness cost varies between strains from different regions [92–94]. Natural substrates of other members of the drug/metabolite transporters include amino acids, weak bases and organic cations [90]. Additionally, it has been shown that CRT homologs in *A. thaliana* transport glutathione. Similar physiological activities could be imagined for PfCRT [95,96].

Several biochemical studies of the parasitic DV, comparing parasites expressing a mutant or a wild type *pfcrt* allele, demonstrated that chloroquine accumulates within the DV and that parasites with mutant PfCRT accumulate less chloroquine than parasites expressing wild type PfCRT [97,98]. The most plausible explanation for this difference in accumulation is that chloroquine resistant parasites can export chloroquine via active transport [99,100]. Interestingly, by heterologous expression of *pfcrt* variants in *Xenopus* oocytes and *D. discoideum*, it has been shown that mutant PfCRT is involved in chloroquine transport while wild type PfCRT is not [39,101]. Wild type and mutant PfCRT might therefore have different drug transport properties.

The involvement of *pfcrt* polymorphisms in chloroquine resistance, in particular the K76T mutation, was established by drug susceptibility experiments (testing parasite strains that were manipulated by allelic exchange and harbored different *pfcrt* alleles [41,102]), and further validated as a strong marker for predicting chloroquine treatment response [103,104]. Combining bioinformatic structure predictions with both biochemical and cell assay data supports the hypothesis that the K76T mutation is located within the first predicted transmembrane spanning domain. This confers chloroquine resistance by exchanging a positively charged residue with an uncharged amino acid, which could allow the active efflux of diprotonated chloroquine out of the DV [90]. Parasites harboring the K76T mutation possess at least three additional mutations within the gene, which possibly compensate for a loss of function caused by the initial K76T mutation. These mutations manifest as a wide variety of PfCRT haplotypes, potentially fine-tuning transport activity and/or specificity. At least 19 additional polymorphic residues have been identified in chloroquine-resistant clinical isolates and parasites subjected to selective drug pressure in vitro [105]. PfCRT haplotypes also influence susceptibility to other antimalarial drugs, including amodiaquine, quinine and lumefantrine [63,104,106]. While amodiaquine and quinine show cross-resistance with chloroquine, mediated primarily by 76T, lumefantrine displays an inverse cross-resistance, with the wild type K76 leading to a reduced susceptibility to lumefantrine. Additionally, it has been suggested that the specific haplotype of amino acids 72–76 further modifies the strength of resistance to amodiaquine [44]. The PfCRT K76T polymorphism is a validated marker for chloroquine resistance in the field and should also be considered as an indicator for amodiaquine resistance.

It has also been shown, that PfCRT is phosphorylated at the residues S33, S411 and T416. Phosphorylation of these amino acids plays an important role in trafficking PfCRT to the DV, which might have additional roles in regulating the transport activity or specificity of the protein [107].

### 4.2. PfMDR1

The *P. falciparum* multidrug resistance transporter 1 (*pfmdr1*) was originally identified by a candidate gene approach in an attempt to identify the determinant of chloroquine resistance searching for the homolog of the multidrug resistance (MDR) transporter family, which is associated with drug resistance in

mammalian tumor cells [108]. The gene, present on chromosome 5, encodes an ATP-binding cassette (ABC) protein of 1419 amino acid and 162 kDa (PFE1150w), which consists of 12 membrane-spanning helices with N- and C-termini predicted to extrude into the cytosol [108,109]. It has been demonstrated that PfMDR1 resides, like PfCRT, within the membrane of the DV [110].

The endogenous function of MDR-like proteins in other organisms consists of the translocation of a variety of substrates including sugars, amino acids, peptides, proteins, metals, inorganic ions, toxins and antibiotics across cellular membranes [111]. Mutations in MDR transporters in mammalian cancer cells lead to a decreased intracellular drug accumulation, increased drug efflux, and cross-resistance to structurally unrelated drugs. The MDR phenotype is often mediated by gene amplification, resulting in overexpression of the protein [111].

PfMDR1 has been shown to import the fluorophore Fluo-4 into the parasitic DV [51], mediated by a functional ATP-binding cassette [112]. Therefore, PfMDR1 might act as a general importer, functioning to sequester toxic metabolites and drugs into the DV. It might also indirectly influence drug flux by affecting intracellular ion gradients, such as chloride ions, or pH [113]. Functional expression studies of PfMDR1 in *Xenopus laevis* oocytes have shown that this protein transports antimalarial drugs, with wild type PfMDR1 transporting quinine and chloroquine but not halofantrine; in contrast, mutant PfMDR1 transports halofantrine but not quinine or chloroquine [114].

From analysis of field isolates, five amino acid positions (86, 184, 1034, 1042 and 1246) have been reported to influence susceptibilities to lumefantrine, artemisinin, quinine, mefloquine, halofantrine and chloroquine [31,61,115,116]. Additionally, amplification of PfMDR1 is associated with reduced susceptibility to lumefantrine, artemisinin, quinine, mefloquine, and halofantrine [52,117] and deamplification of PfMDR1 leads to an increase in chloroquine resistance [42]. Interestingly, amplification of *pfmdr1* also confers a fitness cost to the parasite in in vitro culturing experiments [117,118].

Additionally, evidence suggests that PfMDR1 is phosphorylated [119]. Mammalian MDR transporter homologs, when phosphorylated display altered transport activity [119]. Thus, the phosphorylation status of PfMDR1 could produce a similar alteration of transport.

### 4.3. PfMRP

The multidrug resistance-associated protein (PfMRP) belongs, like PfMDR1, to the family of ATP-binding cassette (ABC) proteins. More specifically, it belongs to the ABC transporter C subfamily, with members in other organisms known to transport anions like glutathione (GSH), glucoronate, sulfate conjugates, and various drugs [120]. *Pfmrp*, located on chromosome 1, encodes an 1822 amino acid protein of 214 kDa, which localizes to the plasma membrane and membrane-bound vesicles within the parasite in asexual and sexual erythrocytic stages (PFA0590w) [120,121]. The protein is not essential in the blood stages, but genetic disruption leads to increased parasite susceptibility to several antimalarial drugs like chloroquine, quinine, artemisinin, piperaquine and primaquine and accumulates more GSH, chloroquine and quinine [121]. Additionally, the lack of expression of PfMRP leads to a fitness cost of the parasite in in vitro culture at parasitemias above 5%, which might be due to a an impaired transport of toxic compounds out of the parasite. There is some evidence from linkage studies based on in vitro drug susceptibility assays with field isolates that point mutations (Y191H and A437S) reduce susceptibility to chloroquine and quinine [122]. In summary, PfMRP modifies drug responses but is not a major determinant in resistance to the currently used drugs and it was hypothesized that PfMRP acts, possibly in concert with other transporters, to efflux drugs and other metabolites out of the parasite [121].

### 4.4. PfNHE

The *P. falciparum* $Na^+/H^+$ exchanger (*pfnhe*) is a candidate gene within a locus on chromosome 13 that was identified during a search for loci associated with quinine resistance in the progeny of the Dd2 × HB3 genetic cross [123]. PfNHE is a 1920 amino acids protein with an approximate molecular mass of 226 kDa (PF13_0019) and is predicted to have 12 transmembrane domains and to be localized to the parasitic plasma membrane [124]. The function of PfNHE has not been fully determined, but it is speculated that it actively effluxes protons to maintain a pH of 7.4 within the parasite, countering acidification by anaerobic glycolysis, the parasite's main source of energy [125]. PfNHE contains three microsatellite regions and the increase of DNNND repeat number in microsatellite ms4670 has been associated with decreased quinine susceptibility, as demonstrated in some studies [126,127] but not in others [29,128]. The inconsistent results concerning the association of PfNHE mutations with quinine resistance indicate either another gene in close physical proximity of *pfnhe* being responsible for the reduction in quinine susceptibility or PfNHE requiring other additional genetic factors for mediating quinine resistance. Because of this, the DNNND repeat number may be a valid marker for quinine resistance in some genetic backgrounds, but not others.

### 4.5. Folate pathway

The folate pathway is a potent drug target in rapidly proliferating cells, like cancer cells and microorganisms. Resulting from this observation, drugs targeting this pathway in *Plasmodium* have been applied very successfully until the emergence of resistant parasites. The folate pathway provides the parasite with cofactors that are essential for the production of pyrimidines for DNA replication and the metabolism of several amino acids. Two enzymes, dihydropteroate synthase (PfDHPS, PF08_0095) and dihydrofolate reductase activity of the bifunctional enzyme, dihydrofolate reductase-thymidylate synthase (PfDHFR-TS, PFD0830w) are currently targeted by antimalarial drugs. PfDHPS is involved in producing a folate precursor and is inhibited by the sulfur-based drugs sulfadoxine and dapsone. PfDHFR is responsible for reducing dihydrofolate into tetrahydrofolate and its function can be impaired by the action of the antifolate drugs pyrimethamine and cycloguanil, the bio-activated metabolite of proguanil [129]. Resistance to this safe and affordable combination therapy sulfadoxine–pyrimethamine (SP, also known as Fansidar) has emerged in the late 1980s [130] and is now wide-spread with point mutations in both *pfdhfr* and *pfdhps* implicated in resistance. Important polymorphisms in PfDHFR, conferring resistance to pyrimethamine, are 108D and 164L, with 51I and 59R further modulating the strength of resistance, in addition to amplification of PfDHFR [131–133]. Resistance to sulfadoxine is strongly associated with amino acids 437G and 581G, with 436A, 540E, and 613S having enhancing effects [133]. Some evidence exists that mutations in PfDHFR also might confer a fitness cost to the parasite [134].

### 4.6. Cytochrome $bc_1$ complex

The cytochrome $bc_1$ complex catalyzes the transfer of electrons from ubiquinol to cytochrome *c*, which is coupled to the translocation of protons across the inner mitochondrial membrane, thereby maintaining the membrane potential of mitochondria used to produce ATP by an ATP synthase [135]. The antimalarial drug

atovaquone can inhibit the parasitic cytochrome $bc_1$ complex by causing a collapse in the mitochondrial membrane potential, which is lethal for the parasite [135]. Several mutations within the cytochrome *b* gene (*cytb*, mal_mito_3) can lead to atovaquone resistance, with most mutations altering the ubiquinol binding site of the protein [74,136]. This site is highly conserved across phyla. Studies with yeast and transgenic parasites support the theory that atovaquone binds to the ubiquinol binding site, thereby disrupting the electron transfer chain [73,137,138]. It has been hypothesized that in the absence of atovaquone drug pressure the mutations in the ubiquinol binding sites might confer a fitness cost to the parasite [135]. Indeed, a double mutation in this conserved protein (M133I and G280D) results in 5–9% fitness cost according to cell culture experiments [138].

## 5. Drug resistance in *P. vivax*

Despite causing an estimated 72–80 million malaria infections yearly, *P. vivax* has not received as much public and scientific attention as *P. falciparum* [5]. One of the main reasons for this is that *P. vivax* usually produces less severe symptoms. Nevertheless, *P. vivax* leads to a disabling disease that can be fatal, and exacts a similar economic burden as falciparum malaria. Additionally, severity of the disease caused by *P. vivax* recently is increasing in some parts of the world and the development of drug resistance could result in an expansion of this debilitating and sometimes deadly infection [139].

To assess drug resistance in *P. vivax* in the field, results from clinical studies and a limited number of laboratory studies using modified drug susceptibility assays, which are recommended by the WHO for *P. falciparum*, have been used [140].

The standard treatment for *P. vivax* infections consists of chloroquine for the treatment of the blood stages and primaquine to eliminate the hypnozoites (or dormant liver stages) that can cause disease relapses [1]. It is apparent now that chloroquine resistance has emerged and is increasing in prevalence, especially in Papua New Guinea and some parts of Indonesia, but there are also reported cases of chloroquine resistant *P. vivax* in Southeast Asia, South America and Africa [139]. For areas with chloroquine resistant vivax malaria the WHO recommends treatment with ACTs, which have proven to be effective also against *P. vivax* in several clinical trials [1]. While chloroquine resistance arose 10 years after its introduction, the cases of chloroquine resistance in *P. vivax* were not reported until 30 years of usage in *P. vivax* treatment. It has been hypothesized that the delay in the emergence of chloroquine resistance is based on a more complicated multigenic mechanism of resistance in *P. vivax* [141]. Another factor, which likely contributed to the delay in chloroquine resistance in *P. vivax*, is the susceptibility of *P. vivax* gametocytes to chloroquine, unlike those of *P. falciparum*, which would decrease the transmission rate [141]. Also, evidence indicates that the immune system is more capable of controlling *P. vivax* infections, thus reducing the disease burden without the requirement for as much chemotherapeutic intervention [141].

The search for drug resistance markers in *P. vivax* has been based on the examination of the known resistance determinants in *P. falciparum*. PvMDR1, the homolog of PfMDR1, has been demonstrated to modulate chloroquine susceptibility [140,142,143]. Of particular importance is the Y976F mutation, which is strongly associated with resistance in the Western Pacific regions. Unlike in *P. falciparum*, the *P. vivax* ortholog of PfCRT, PvCRT, has not been demonstrated to contribute significantly to chloroquine resistance (both sensitive and resistant vivax parasites carry K76), even with high protein conservation and suspected performance of similar biological functions [144].

In areas where *P. vivax* and *P. falciparum* are co-endemic, therapy is usually administered for the treatment of falciparum malaria, either because of misdiagnosis or due to a diagnosed mixed infection (in which case primaquine is also administered to treat the *P. vivax* hypnozoite stage). This results in exposure of *P. vivax* to additional antimalarial drugs, which likely exert some drug selection pressure on *P. vivax* parasites. Indeed, mutations in the ortholog enzymes targeted by sulfadoxine–pyrimethamine, PvDHFR and PvDHPS, have been found in areas where *P. falciparum* infections have been treated with this antifolate drug combination [145]. In addition, in regions where mefloquine has been used for the treatment of falciparum malaria, there is an increased prevalence of *pvmdr1* copy number variation, suggesting that a similar mechanism might decrease the susceptibility to mefloquine in both *Plasmodia* species [143]. Also, preliminary evidence suggests that altered susceptibility to artesunate is caused by mutations in *pvmdr1* and its amplification status [143].

## 6. Conclusion

With the emergence of drug resistance to the artemisinin derivatives, often referred to as the last stronghold in malaria chemotherapeutical treatment, it is of critical importance to implement antimalarial drug policies to contain, and hopefully curtail the spread of resistance. Failure to do so would lead to a tragic setback in the current efforts to eliminate malaria, and achieved reductions in malaria-related morbidity and mortality.

Several drug regimen strategies can be applied to maximize the lifespan of the currently used antimalarials, including the use of multiple first-line treatments, which would lead to varying drug selection pressure on local parasite populations, and therefore decrease the emergence and spread of parasites resistant to any one particular antimalarial [146]. One important approach that has already been employed is the use of combination therapies, which decreases the selection of drug-resistant parasites by the action of the partner drugs. Critical to this strategy is the evaluation of preexisting resistance to the partner drug used (or proposed) for any given region, since most have been utilized previously as monotherapy, with the notable exception of lumefantrine. One interesting strategy that should be further investigated is the use of a combination of drugs that exert opposing drug resistance pressures, for example chloroquine and lumefantrine [63]. Adherence to the full treatment regimen is essential to delay the emergence of drug-resistant parasites and ensure adequate treatment of individual infections. To address this, ACTs should be co-formulated to make certain that all drugs in the combination are administered simultaneously, along with public education and training of community health workers to increase compliance. Another previously proposed strategy to extend the useful lifespan of the limited antimalarial repertoire currently available is the reintroduction of drugs after the prevalence of resistant parasites has waned. This approach is based on the assumption that drug-resistant parasites would carry a fitness cost, compared to sensitive parasites, and would therefore be outcompeted without the continued advantage during drug selection pressure [92]. This would require that compensatory mutations had not already increased the fitness of the resistant parasites. The re-selection or importation of drug-resistant parasites after re-implementation of an antimalarial should therefore be vigilantly monitored.

Current tools, including long-lasting insecticide treated bed nets, indoor residual spraying and effective, rapid case management have clearly demonstrated a benefit in the reduction of malaria [1]. Full implementation of these, along with other transmission interventions currently being developed (transmission blocking

drugs or vaccines), can pare down the spread of drug resistance after it has emerged. Combined with effective, rational drug treatment policies, existing tools can further reduce malaria morbidity and mortality worldwide to levels that have never been achieved. This will, however, require sustained financial and political support and an understanding of the genetic mechanisms and pharmacological factors that impact treatment efficacy, with a continuation of efforts to monitor and address the emergence of drug-resistant parasites. Together, these efforts will yield an important reduction in the global malaria burden and provide a significant benefit to global public health.

## Acknowledgements

We thank Marcel Deponte and Sophie Adjalley for their constructive comments and suggestions on the manuscript. This work was supported by the Deutsche Forschungsgemeinschaft under the portfolio of the Special Research Focus "Control of Tropical Infectious Diseases". M.L. is a member of the German Excellence Cluster "Cellnetworks" and the European Network of Excellence "EviMalaR".

## References

[1] World Health Organization (2010) World Malaria Report 2010, WHO Press. pp. 1–152.
[2] World Health Organization (2010) World Health Statistics 2010, WHO Press.
[3] Bray, R.S. and Garnham, P.C. (1982) The life-cycle of primate malaria parasites. Br. Med. Bull. 38, 117–122.
[4] Cox-Singh, J. et al. (2008) *Plasmodium knowlesi* malaria in humans is widely distributed and potentially life threatening. Clin. Infect. Dis. 46, 165–171.
[5] Price, R.N., Tjitra, E., Guerra, C.A., Yeung, S., White, N.J. and Anstey, N.M. (2007) Vivax malaria: neglected and not benign. Am. J. Trop. Med. Hyg. 77, 79–87.
[6] Hay, S., Guerra, C., Tatem, A., Noor, A. and Snow, R. (2004) The global distribution and population at risk of malaria: past, present, and future. Lancet Infect. Dis. 4, 327–336.
[7] Nájera, J.A., González-Silva, M. and Alonso, P.L. (2011) Some lessons for the future from the Global Malaria Eradication Programme (1955–1969). PLoS Med. 8, e1000412.
[8] Trape, J.F. (2001) The public health impact of chloroquine resistance in Africa. Am. J. Trop. Med. Hyg. 64, 12–17.
[9] Clyde, D.F. and Shute, G.T. (1957) Resistance of *Plasmodium falciparum* in Tanganyika to pyrimethamine administered at weekly intervals. Trans. R. Soc. Trop. Med. Hyg. 51, 505–513.
[10] Egan, T.J. and Kaschula, C.H. (2007) Strategies to reverse drug resistance in malaria. Curr. Opin. Infect. Dis. 20, 598–604.
[11] Dondorp, A.M. et al. (2009) Artemisinin resistance in *Plasmodium falciparum* malaria. N. Engl. J. Med. 361, 455–467.
[12] Anderson, T.J. et al. (2010) High heritability of malaria parasite clearance rate indicates a genetic basis for artemisinin resistance in western Cambodia. J. Infect. Dis. 201, 1326–1330.
[13] Sachs, J. and Malaney, P. (2002) The economic and social burden of malaria. Nature 415, 680–685.
[14] Paget-McNicol, S. and Saul, A. (2001) Mutation rates in the dihydrofolate reductase gene of *Plasmodium falciparum*. Parasitology 122, 497–505.
[15] Sniegowski, P.D., Gerrish, P.J., Johnson, T. and Shaver, A. (2000) The evolution of mutation rates: separating causes from consequences. BioEssays 22, 1057–1066.
[16] Rathod, P.K., McErlean, T. and Lee, P.C. (1997) Variations in frequencies of drug resistance in *Plasmodium falciparum*. Proc. Natl. Acad. Sci. USA 94, 9389–9393.
[17] Levin, B.R., Perrot, V. and Walker, N. (2000) Compensatory mutations, antibiotic resistance and the population genetics of adaptive evolution in bacteria. Genetics 154, 985.
[18] Hastings, I.M., Paget-McNicol, S. and Saul, A. (2004) Can mutation and selection explain virulence in human *P. falciparum* infections? Malaria J. 3, 2.
[19] Müller, O. (2011) Malaria in Africa. Challenges for Control and Elimination in the 21st Century, Peter Lang Publication, Frankfurt am Main, Germany.
[20] Stepniewska, K. and White, N.J. (2008) Pharmacokinetic determinants of the window of selection for antimalarial drug resistance. Antimicrob. Agents Chemother. 52, 1589–1596.
[21] Hastings, I.M. and Watkins, W.M. (2006) Tolerance is the key to understanding antimalarial drug resistance. Trends Parasitol. 22, 71–77.
[22] Anderson, T.J. and Roper, C. (2005) The origins and spread of antimalarial drug resistance: lessons for policy makers. Acta Trop. 94, 269–280.
[23] Färnert, A. et al. (2009) Transmission-dependent tolerance to multiclonal *Plasmodium falciparum* infection. J. Infect. Dis. 200, 1166–1175.
[24] Okell, L.C., Drakeley, C.J., Ghani, A.C., Bousema, T. and Sutherland, C.J. (2008) Reduction of transmission from malaria patients by artemisinin combination therapies: a pooled analysis of six randomized trials. Malaria J. 7, 125.
[25] White, N.J. (2008) The role of anti-malarial drugs in eliminating malaria. Malaria J. 7 (Suppl. 1), S8.
[26] Hastings, I.M. (2006) Gametocytocidal activity in antimalarial drugs speeds the spread of drug resistance. Trop. Med. Int. Health 11, 1206–1217.
[27] Butler, A.R., Khan, S. and Ferguson, E. (2010) A brief history of malaria chemotherapy. J. R. Coll. Physicians Edinb. 40, 172–177.
[28] Meng, H., Zhang, R., Yang, H., Fan, Q., Su, X., Miao, J., Cui, L. and Yang, Z. (2010) In vitro sensitivity of *Plasmodium falciparum* clinical isolates from the China–Myanmar border area to quinine and association with polymorphism in the $Na^+/H^+$ exchanger. Antimicrob. Agents Chemother. 54, 4306–4313.
[29] Briolant, S. et al. (2011) In vitro susceptibility to quinine and microsatellite variations of the *Plasmodium falciparum* $Na^+/H^+$ exchanger (Pfnhe-1) gene: the absence of association in clinical isolates from the Republic of Congo. Malaria J. 10, 37.
[30] Fitch, C.D. (2004) Ferriprotoporphyrin IX, phospholipids, and the antimalarial actions of quinoline drugs. Life Sci. 74, 1957–1972.
[31] Sidhu, A.B., Valderramos, S.G. and Fidock, D.A. (2005) *Pfmdr1* mutations contribute to quinine resistance and enhance mefloquine and artemisinin sensitivity in *Plasmodium falciparum*. Mol. Microbiol. 57, 913–926.
[32] Nkrumah, L.J. et al. (2009) Probing the multifactorial basis of *Plasmodium falciparum* quinine resistance: evidence for a strain-specific contribution of the sodium-proton exchanger PfNHE. Mol. Biochem. Parasitol. 165, 122–131.
[33] Cooper, R.A. et al. (2002) Alternative mutations at position 76 of the vacuolar transmembrane protein PfCRT are associated with chloroquine resistance and unique stereospecific quinine and quinidine responses in *Plasmodium falciparum*. Mol. Pharmacol. 61, 35–42.
[34] Cooper, R.A., Lane, K.D., Deng, B., Mu, J., Patel, J.J., Wellems, T.E., Su, X. and Ferdig, M.T. (2007) Mutations in transmembrane domains 1, 4 and 9 of the *Plasmodium falciparum* chloroquine resistance transporter alter susceptibility to chloroquine, quinine and quinidine. Mol. Microbiol. 63, 270–282.
[35] AlKadi, H.O. (2007) Antimalarial drug toxicity: a review. Chemotherapy 53, 385–391.
[36] Mita, T., Tanabe, K. and Kita, K. (2009) Spread and evolution of *Plasmodium falciparum* drug resistance. Parasitol. Int. 58, 201–209.
[37] Wellems, T.E. and Plowe, C.V. (2001) Chloroquine-resistant malaria. J. Infect. Dis. 184, 770–776.
[38] Egan, T.J. (2008) Haemozoin formation. Mol. Biochem. Parasitol. 157, 127–136.
[39] Martin, R.E., Marchetti, R.V., Cowan, A.I., Howitt, S.M., Bröer, S. and Kirk, K. (2009) Chloroquine transport via the malaria parasite's chloroquine resistance transporter. Science (New York, NY) 325, 1680–1682.
[40] Zhang, J., Krugliak, M. and Ginsburg, H. (1999) The fate of ferriprotorphyrin IX in malaria infected erythrocytes in conjunction with the mode of action of antimalarial drugs. Mol. Biochem. Parasitol. 99, 129–141.
[41] Sidhu, A.B., Verdier-Pinard, D. and Fidock, D.A. (2002) Chloroquine resistance in *Plasmodium falciparum* malaria parasites conferred by *pfcrt* mutations. Science (New York, NY) 298, 210–213.
[42] Barnes, D.A., Foote, S.J., Galatis, D., Kemp, D.J. and Cowman, A. (1992) Selection for high-level chloroquine resistance results in deamplification of the *pfmdr1* gene and increased sensitivity to mefloquine in *Plasmodium falciparum*. EMBO J. 11, 3067–3075.
[43] Osman, M.E., Mockenhaupt, F.P., Bienzle, U., Elbashir, M.I. and Giha, H.A. (2007) Field-based evidence for linkage of mutations associated with chloroquine (*pfcrt*/*pfmdr1*) and sulfadoxine–pyrimethamine (pfdhfr/pfdhps) resistance and for the fitness cost of multiple mutations in *P. falciparum*. Infect. Genet. Evol. 7, 52–59.
[44] Sá, J.M., Twu, O., Hayton, K., Reyes, S., Fay, M.P., Ringwald, P. and Wellems, T.E. (2009) Inaugural article: geographic patterns of *Plasmodium falciparum* drug resistance distinguished by differential responses to amodiaquine and chloroquine. Proc. Natl. Acad. Sci. USA 106, 18883–18889.
[45] Hayeshi, R., Masimirembwa, C., Mukanganyama, S. and Ungell, A.L. (2008) Lysosomal trapping of amodiaquine: impact on transport across intestinal epithelia models. Biopharm. Drug Dispos. 29, 324–334.
[46] Foley, M. and Tilley, L. (1998) Quinoline antimalarials: mechanisms of action and resistance and prospects for new agents. Pharmacol. Ther. 79, 55–87.
[47] Trenholme, C.M., Williams, R.L., Desjardins, R.E., Frischer, H., Carson, P.E., Rieckmann, K.H. and Canfield, C.J. (1975) Mefloquine (WR 142,490) in the treatment of human malaria. Science (New York, NY) 190, 792–794.
[48] Cowman, A., Galatis, D. and Thompson, J.K. (1994) Selection for mefloquine resistance in *Plasmodium falciparum* is linked to amplification of the *pfmdr1* gene and cross-resistance to halofantrine and quinine. Proc. Natl. Acad. Sci. USA 91, 1143–1147.
[49] Fitch, C.D., Chan, R.L. and Chevli, R. (1979) Chloroquine resistance in malaria: accessibility of drug receptors to mefloquine. Antimicrob. Agents Chemother. 15, 258–262.
[50] Eastman, R.T. and Fidock, D.A. (2009) Artemisinin-based combination therapies: a vital tool in efforts to eliminate malaria. Nat. Rev. Microbiol. 7, 864–874.
[51] Rohrbach, P. et al. (2006) Genetic linkage of *pfmdr1* with food vacuolar solute import in *Plasmodium falciparum*. EMBO J. 25, 3000–3011.
[52] Sidhu, A.B., Uhlemann, A.C., Valderramos, S.G., Valderramos, J.C., Krishna, S. and Fidock, D.A. (2006) Decreasing *pfmdr1* copy number in *plasmodium*

*falciparum* malaria heightens susceptibility to mefloquine, lumefantrine, halofantrine, quinine, and artemisinin. J. Infect. Dis. 194, 528–535.
[53] Warhurst, D.C., Craig, J.C., Adagu, I.S., Guy, R.K., Madrid, P.B. and Fivelman, Q.L. (2007) Activity of piperaquine and other 4-aminoquinoline antiplasmodial drugs against chloroquine-sensitive and resistant blood-stages of *Plasmodium falciparum*. Role of beta-haematin inhibition and drug concentration in vacuolar water- and lipid-phases. Biochem. Pharmacol. 73, 1910–1926.
[54] Raynes, K. (1999) Bisquinoline antimalarials: their role in malaria chemotherapy. Int. J. Parasitol. 29, 367–379.
[55] Fivelman, Q.L., Adagu, I.S. and Warhurst, D.C. (2007) Effects of piperaquine, chloroquine, and amodiaquine on drug uptake and of these in combination with dihydroartemisinin against drug-sensitive and -resistant *Plasmodium falciparum* strains. Antimicrob. Agents Chemother. 51, 2265–2267.
[56] Davis, T.M., Hung, T.Y., Sim, I.K., Karunajeewa, H.A. and Ilett, K.F. (2005) Piperaquine: a resurgent antimalarial drug. Drugs 65, 75–87.
[57] Muangnoicharoen, S., Johnson, D.J., Looareesuwan, S., Krudsood, S. and Ward, S.A. (2009) Role of known molecular markers of resistance in the antimalarial potency of piperaquine and dihydroartemisinin in vitro. Antimicrob. Agents Chemother. 53, 1362–1366.
[58] Ezzet, F., Mull, R. and Karbwang, J. (1998) Population pharmacokinetics and therapeutic response of CGP 56697 (artemether + benflumetol) in malaria patients. Br. J. Clin. Pharmacol. 46, 553–561.
[59] Ashley, E.A. et al. (2007) How much fat is necessary to optimize lumefantrine oral bioavailability? Trop. Med. Int. Health 12, 195–200.
[60] Sisowath, C., Ferreira, P.E., Bustamante, L.Y., Dahlström, S., Mårtensson, A., Björkman, A., Krishna, S. and Gil, J.P. (2007) The role of *pfmdr1* in *Plasmodium falciparum* tolerance to artemether–lumefantrine in Africa. Trop. Med. Int. Health 12, 736–742.
[61] Sisowath, C., Strömberg, J., Mårtensson, A., Msellem, M., Obondo, C., Björkman, A. and Gil, J.P. (2005) In vivo selection of *Plasmodium falciparum pfmdr1* 86N coding alleles by artemether–lumefantrine (Coartem). J. Infect. Dis. 191, 1014–1017.
[62] Price, R.N. et al. (2006) Molecular and pharmacological determinants of the therapeutic response to artemether–lumefantrine in multidrug-resistant *Plasmodium falciparum* malaria. Clin. Infect. Dis. 42, 1570–1577.
[63] Sisowath, C., Petersen, I., Veiga, M.I., Mårtensson, A., Premji, Z., Björkman, A., Fidock, D.A. and Gil, J.P. (2009) In Vivo Selection of *Plasmodium falciparum* parasites carrying the chloroquine-susceptible *pfcrt* K76 allele after treatment with artemether–lumefantrine in Africa. J. Infect. Dis. 199, 750–757.
[64] Edwards, G., McGrath, C.S., Ward, S.A., Supanaranond, W., Pukrittayakamee, S., Davis, T.M. and White, N.J. (1993) Interactions among primaquine, malaria infection and other antimalarials in Thai subjects. Br. J. Clin. Pharmacol. 35, 193–198.
[65] Wells, T.N., Burrows, J.N. and Baird, J.K. (2010) Targeting the hypnozoite reservoir of *Plasmodium vivax*: the hidden obstacle to malaria elimination. Trends Parasitol. 26, 145–151.
[66] Beutler, E., Duparc, S. and Group, G.P.D.W. (2007) Glucose-6-phosphate dehydrogenase deficiency and antimalarial drug development. Am. J. Trop. Med. Hyg. 77, 779–789.
[67] Egan, T.J. (2006) Chloroquine and primaquine: combining old drugs as a new weapon against falciparum malaria? Trends Parasitol. 22, 235–237.
[68] Bray, P.G., Deed, S., Fox, E., Kalkanidis, M., Mungthin, M., Deady, L.W. and Tilley, L. (2005) Primaquine synergises the activity of chloroquine against chloroquine-resistant *P. falciparum*. Biochem. Pharmacol. 70, 1158–1166.
[69] Sanchez, C.P., McLean, J.E., Stein, W. and Lanzer, M. (2004) Evidence for a substrate specific and inhibitable drug efflux system in chloroquine resistant *Plasmodium falciparum* strains. Biochemistry 43, 16365–16373.
[70] Srivastava, I.K., Rottenberg, H. and Vaidya, A.B. (1997) Atovaquone, a broad spectrum antiparasitic drug, collapses mitochondrial membrane potential in a malarial parasite. J. Biol. Chem. 272, 3961–3966.
[71] Hughes, W.T. et al. (1991) Safety and pharmacokinetics of 566C80, a hydroxynaphthoquinone with anti-*Pneumocystis carinii* activity: a phase I study in human immunodeficiency virus (HIV)-infected men. J. Infect. Dis. 163, 843–848.
[72] Hudson, A.T. et al. (1991) 566C80: a potent broad spectrum anti-infective agent with activity against malaria and opportunistic infections in AIDS patients. Drugs Exp. Clin. Res. 17, 427–435.
[73] Painter, H.J., Morrisey, J.M., Mather, M.W. and Vaidya, A.B. (2007) Specific role of mitochondrial electron transport in blood-stage *Plasmodium falciparum*. Nature 446, 88–91.
[74] Gil, J.P. et al. (2003) Detection of atovaquone and Malarone resistance conferring mutations in *Plasmodium falciparum* cytochrome *b* gene (*cytb*). Mol. Cell. Probes 17, 85–89.
[75] Srivastava, I.K. and Vaidya, A.B. (1999) A mechanism for the synergistic antimalarial action of atovaquone and proguanil. Antimicrob. Agents Chemother. 43, 1334–1339.
[76] Uhlemann, A.C. and Krishna, S. (2005) Antimalarial multi-drug resistance in Asia: mechanisms and assessment. Curr. Top. Microbiol. Immunol. 295, 39–53.
[77] Luzzatto, L. (2010) The rise and fall of the antimalarial Lapdap: a lesson in pharmacogenetics. Lancet 376, 739–741.
[78] Bray, P.G., Ward, S.A. and O'Neill, P.M. (2005) Quinolines and artemisinin: chemistry, biology and history. Curr. Top. Microbiol. Immunol. 295, 3–38.
[79] Bloland, P.B. (2001) Drug Resistance in Malaria, World Health Organization, Geneva.
[80] Jambou, R. et al. (2005) Resistance of *Plasmodium falciparum* field isolates to in-vitro artemether and point mutations of the SERCA-type PfATPase6. Lancet 366, 1960–1963.
[81] Hunt, P. et al. (2007) Gene encoding a deubiquitinating enzyme is mutated in artesunate- and chloroquine-resistant rodent malaria parasites. Mol. Microbiol. 65, 27–40.
[82] Imwong, M. et al. (2010) Exploring the contribution of candidate genes to artemisinin resistance in *Plasmodium falciparum*. Antimicrob. Agents Chemother. 54, 2886–2892.
[83] Chavchich, M., Gerena, L., Peters, J., Chen, N., Cheng, Q. and Kyle, D.E. (2010) Role of *pfmdr1* amplification and expression in induction of resistance to artemisinin derivatives in *Plasmodium falciparum*. Antimicrob. Agents Chemother. 54, 2455–2464.
[84] Witkowski, B., Lelievre, J., Lopez Barragan, M.J., Laurent, V., Su, X., Berry, A. and Benoit-Vical, F. (2010) Increased tolerance to artemisinin in *Plasmodium falciparum* is mediated by a quiescence mechanism. Antimicrob. Agents Chemother. 54, 1872.
[85] Müller, O., Sié, A., Meissner, P., Schirmer, R.H. and Kouyaté, B. (2009) Artemisinin resistance on the Thai–Cambodian border. Lancet 374, 1419.
[86] Gardner, M.J. et al. (2002) Genome sequence of the human malaria parasite *Plasmodium falciparum*. Nature 419, 498–511.
[87] Meissner, M., Breinich, M.S., Gilson, P.R. and Crabb, B.S. (2007) Molecular genetic tools in *Toxoplasma* and *Plasmodium*: achievements and future needs. Curr. Opin. Microbiol. 10, 349–356.
[88] Su, X., Kirkman, L.A., Fujioka, H. and Wellems, T.E. (1997) Complex polymorphisms in an approximately 330 kDa protein are linked to chloroquine-resistant *P. falciparum* in Southeast Asia and Africa. Cell 91, 593–603.
[89] Fidock, D.A. et al. (2000) Mutations in the *P. falciparum* digestive vacuole transmembrane protein PfCRT and evidence for their role in chloroquine resistance. Mol. Cell 6, 861–871.
[90] Martin, R.E. and Kirk, K. (2004) The malaria parasite's chloroquine resistance transporter is a member of the drug/metabolite transporter superfamily. Mol. Biol. Evol. 21, 1938–1949.
[91] Waller, K.L. et al. (2003) Chloroquine resistance modulated in vitro by expression levels of the *Plasmodium falciparum* chloroquine resistance transporter. J. Biol. Chem. 278, 33593–33601.
[92] Kublin, J.G. et al. (2003) Reemergence of chloroquine-sensitive *Plasmodium falciparum* malaria after cessation of chloroquine use in Malawi. J. Infect. Dis. 187, 1870–1875.
[93] Nguyen, M.H. et al. (2003) Treatment of uncomplicated falciparum malaria in southern Vietnam: can chloroquine or sulfadoxine–pyrimethamine be reintroduced in combination with artesunate? Clin. Infect. Dis. 37, 1461–1466.
[94] Yang, H., Yang, Y., Yang, P., Li, X., Gao, B., Zhang, Z., Yang, Z. and Cui, L. (2008) Monitoring *Plasmodium falciparum* chloroquine resistance in Yunnan Province, China, 1981–2006. Acta Trop. 108, 44–49.
[95] Meierjohann, S., Walter, R.D. and Müller, S. (2002) Regulation of intracellular glutathione levels in erythrocytes infected with chloroquine-sensitive and chloroquine-resistant *Plasmodium falciparum*. Biochem. J. 368, 761–768.
[96] Maughan, S. et al. (2010) Plant homologs of the *Plasmodium falciparum* chloroquine-resistance transporter, PfCRT, are required for glutathione homeostasis and stress responses. Proc. Natl. Acad. Sci. 1, 6.
[97] Sanchez, C.P., McLean, J.E., Rohrbach, P., Fidock, D.A., Stein, W.D. and Lanzer, M. (2005) Evidence for a *pfcrt*-associated chloroquine efflux system in the human malarial parasite *Plasmodium falciparum*. Biochemistry 44, 9862–9870.
[98] Yayon, A., Cabantchik, Z.I. and Ginsburg, H. (1984) Identification of the acidic compartment of *Plasmodium falciparum*-infected human erythrocytes as the target of the antimalarial drug chloroquine. EMBO J. 3, 2695–2700.
[99] Sanchez, C.P., Rohrbach, P., McLean, J.E., Fidock, D.A., Stein, W.D. and Lanzer, M. (2007) Differences in trans-stimulated chloroquine efflux kinetics are linked to PfCRT in *Plasmodium falciparum*. Mol. Microbiol. 64, 407–420.
[100] Sanchez, C.P., Stein, W.D. and Lanzer, M. (2007) Is PfCRT a channel or a carrier? Two competing models explaining chloroquine resistance in *Plasmodium falciparum*. Trends Parasitol. 23, 332–339.
[101] Naudé, B., Brzostowski, J.A., Kimmel, A.R. and Wellems, T.E. (2005) Dictyostelium discoideum expresses a malaria chloroquine resistance mechanism upon transfection with mutant, but not wild-type, *Plasmodium falciparum* transporter PfCRT. J. Biol. Chem. 280, 25596–25603.
[102] Lakshmanan, V. et al. (2005) A critical role for PfCRT K76T in *Plasmodium falciparum* verapamil-reversible chloroquine resistance. EMBO J. 24, 2294–2305.
[103] Djimdé, A. et al. (2001) A molecular marker for chloroquine-resistant falciparum malaria. N. Engl. J. Med. 344, 257–263.
[104] Menard, D., Yapou, F., Manirakiza, A., Djalle, D., Matsika-Claquin, M.D. and Talarmin, A. (2006) Polymorphisms in *pfcrt*, *pfmdr1*, *dhfr* genes and in vitro responses to antimalarials in *Plasmodium falciparum* isolates from Bangui, Central African Republic. Am. J. Trop. Med. Hyg. 75, 381–387.
[105] Cooper, R.A., Hartwig, C.L. and Ferdig, M.T. (2005) *Pfcrt* is more than the *Plasmodium falciparum* chloroquine resistance gene: a functional and evolutionary perspective. Acta Trop. 94, 170–180.
[106] Echeverry, D.F., Holmgren, G., Murillo, C., Higuita, J.C., Björkman, A., Gil, J.P. and Osorio, L. (2007) Short report: polymorphisms in the *pfcrt* and *pfmdr1*

genes of *Plasmodium falciparum* and in vitro susceptibility to amodiaquine and desethylamodiaquine. Am. J. Trop. Med. Hyg. 77, 1034–1038.
[107] Kuhn, Y., Sanchez, C.P., Ayoub, D., Saridaki, T., van Dorsselaer, A. and Lanzer, M. (2009) Trafficking of the phosphoprotein PfCRT to the digestive vacuolar membrane in *Plasmodium falciparum*. Traffic (Copenhagen, Denmark) 11, 236–249.
[108] Foote, S.J., Thompson, J.K., Cowman, A. and Kemp, D.J. (1989) Amplification of the multidrug resistance gene in some chloroquine-resistant isolates of *P. falciparum*. Cell 57, 921–930.
[109] Duraisingh, M. and Cowman, A.F. (2005) Contribution of the *pfmdr1* gene to antimalarial drug-resistance. Acta Trop. 94, 181–190.
[110] Cowman, A., Karcz, S., Galatis, D. and Culvenor, J.G. (1991) A P-glycoprotein homologue of *Plasmodium falciparum* is localized on the digestive vacuole. J. Cell Biol. 113, 1033–1042.
[111] Peel, S.A. (2001) The ABC transporter genes of *Plasmodium falciparum* and drug resistance. Drug Resist. Updat. 4, 66–74.
[112] Karcz, S.R., Galatis, D. and Cowman, A. (1993) Nucleotide binding properties of a P-glycoprotein homologue from *Plasmodium falciparum*. Mol. Biochem. Parasitol. 58, 269–276.
[113] van Es, H.H., Renkema, H., Aerts, H. and Schurr, E. (1994) Enhanced lysosomal acidification leads to increased chloroquine accumulation in CHO cells expressing the *pfmdr1* gene. Mol. Biochem. Parasitol. 68, 209–219.
[114] Sanchez, C.P., Rotmann, A., Stein, W.D. and Lanzer, M. (2008) Polymorphisms within PfMDR1 alter the substrate specificity for anti-malarial drugs in *Plasmodium falciparum*. Mol. Microbiol. 70, 786–798.
[115] Reed, M.B., Saliba, K.J., Caruana, S.R., Kirk, K. and Cowman, A. (2000) Pgh1 modulates sensitivity and resistance to multiple antimalarials in *Plasmodium falciparum*. Nature 403, 906–909.
[116] Pickard, A.L. et al. (2003) Resistance to antimalarials in Southeast Asia and genetic polymorphisms in *pfmdr1*. Antimicrob. Agents Chemother. 47, 2418–2423.
[117] Preechapornkul, P., Imwong, M., Chotivanich, K., Pongtavornpinyo, W., Dondorp, A., Day, N.P.J., White, N.J. and Pukrittayakamee, S. (2009) *Plasmodium falciparum pfmdr1* amplification, mefloquine resistance, and parasite fitness. Antimicrob. Agents Chemother. 53, 1509.
[118] Hayward, R., Saliba, K.J. and Kirk, K. (2005) *Pfmdr1* mutations associated with chloroquine resistance incur a fitness cost in *Plasmodium falciparum*. Mol. Microbiol. 55, 1285–1295.
[119] Lim, A. and Cowman, A. (1993) Phosphorylation of a P-glycoprotein homologue in *Plasmodium falciparum*. Mol. Biochem. Parasitol. 62, 293–302.
[120] Klokouzas, A., Tiffert, T., van Schalkwyk, D., Wu, C.P., van Veen, H.W., Barrand, M.A. and Hladky, S.B. (2004) *Plasmodium falciparum* expresses a multidrug resistance-associated protein. Biochem. Biophys. Res. Commun. 321, 197–201.
[121] Raj, D.K. et al. (2009) Disruption of a *Plasmodium falciparum* multidrug resistance-associated protein (PfMRP) alters its fitness and transport of antimalarial drugs and glutathione. J. Biol. Chem. 284, 7687–7696.
[122] Mu, J., Ferdig, M.T., Feng, X., Joy, D., Duan, J. and Furuya, T. (2003) Multiple transporters associated with malaria parasite responses to chloroquine and quinine. Mol. Microbiol. 49, 977–989.
[123] Ferdig, M.T., Cooper, R.A., Mu, J., Deng, B., Joy, D.A., Su, X.Z. and Wellems, T.E. (2004) Dissecting the loci of low-level quinine resistance in malaria parasites. Mol. Microbiol. 52, 985–997.
[124] Bennett, T.N., Patel, J., Ferdig, M.T. and Roepe, P.D. (2007) *Plasmodium falciparum* $Na^+/H^+$ exchanger activity and quinine resistance. Mol. Biochem. Parasitol. 153, 48–58.
[125] Bosia, A., Ghigo, D., Turrini, F., Nissani, E., Pescarmona, G.P. and Ginsburg, H. (1993) Kinetic characterization of $Na^+/H^+$ antiport of *Plasmodium falciparum* membrane. J. Cell. Physiol. 154, 527–534.
[126] Okombo, J. et al. (2010) In vitro activities of quinine and other antimalarials and pfnhe polymorphisms in *Plasmodium* isolates from Kenya. Antimicrob. Agents Chemother. 54, 3302–3307.
[127] Henry, M. et al. (2009) *Plasmodium falciparum* $Na^+/H^+$ exchanger 1 transporter is involved in reduced susceptibility to quinine. Antimicrob. Agents Chemother. 53, 1926–1930.
[128] Andriantsoanirina, V., Ménard, D., Rabearimanana, S., Hubert, V., Bouchier, C., Tichit, M., Bras, J.L. and Durand, R. (2010) Association of microsatellite variations of *Plasmodium falciparum* $Na^+/H^+$ exchanger (*Pfnhe-1*) gene with reduced in vitro susceptibility to quinine: lack of confirmation in clinical isolates from Africa. Am. J. Trop. Med. Hyg. 82, 782–787.
[129] Hyde, J.E. (2005) Exploring the folate pathway in *Plasmodium falciparum*. Acta Trop. 94, 191–206.
[130] Cowman, A., Morry, M.J., Biggs, B.A., Cross, G.A. and Foote, S.J. (1988) Amino acid changes linked to pyrimethamine resistance in the dihydrofolate reductase-thymidylate synthase gene of *Plasmodium falciparum*. Proc. Natl. Acad. Sci. USA 85, 9109–9113.
[131] Thaithong, S. et al. (2001) *Plasmodium falciparum*: gene mutations and amplification of dihydrofolate reductase genes in parasites grown in vitro in presence of pyrimethamine. Exp. Parasitol. 98, 59–70.
[132] Urdaneta, L., Plowe, C., Goldman, I. and Lal, A.A. (1999) Point mutations in dihydrofolate reductase and dihydropteroate synthase genes of *Plasmodium falciparum* isolates from Venezuela. Am. J. Trop. Med. Hyg. 61, 457–462.
[133] Wernsdorfer, W.H. and Noedl, H. (2003) Molecular markers for drug resistance in malaria: use in treatment, diagnosis and epidemiology. Curr. Opin. Infect. Dis. 16, 553–558.
[134] Fohl, L.M. and Roos, D.S. (2003) Fitness effects of DHFR-TS mutations associated with pyrimethamine resistance in apicomplexan parasites. Mol. Microbiol. 50, 1319–1327.
[135] Barton, V., Fisher, N., Biagini, G.A., Ward, S.A. and O'Neill, P.M. (2010) Inhibiting *Plasmodium* cytochrome $bc_1$: a complex issue. Curr. Opin. Chem. Biol. 14, 440–446.
[136] Korsinczky, M., Chen, N., Kotecka, B., Saul, A., Rieckmann, K. and Cheng, Q. (2000) Mutations in *Plasmodium falciparum* cytochrome *b* that are associated with atovaquone resistance are located at a putative drug-binding site. Antimicrob. Agents Chemother. 44, 2100–2108.
[137] Kessl, J.J. et al. (2003) Molecular basis for atovaquone binding to the cytochrome $bc_1$ complex. J. Biol. Chem. 278, 31312–31318.
[138] Peters, J.M., Chen, N., Gatton, M., Korsinczky, M., Fowler, E.V., Manzetti, S., Saul, A. and Cheng, Q. (2002) Mutations in cytochrome *b* resulting in atovaquone resistance are associated with loss of fitness in *Plasmodium falciparum*. Antimicrob. Agents Chemother. 46, 2435–2441.
[139] Price, R.N., Douglas, N.M. and Anstey, N.M. (2009) New developments in *Plasmodium vivax* malaria: severe disease and the rise of chloroquine resistance. Curr. Opin. Infect. Dis. 22, 430–435.
[140] Suwanarusk, R. et al. (2007) Chloroquine resistant *Plasmodium vivax*: in vitro characterisation and association with molecular polymorphisms. PLoS ONE 2, e1089.
[141] Mendis, K., Sina, B.J., Marchesini, P. and Carter, R. (2001) The neglected burden of *Plasmodium vivax* malaria. Am. J. Trop. Med. Hyg. 64, 97–106.
[142] Russell, B. et al. (2008) Determinants of in vitro drug susceptibility testing of *Plasmodium vivax*. Antimicrob. Agents Chemother. 52, 1040–1045.
[143] Suwanarusk, R. et al. (2008) Amplification of pvmdr1 associated with multidrug-resistant *Plasmodium vivax*. J. Infect. Dis. 198, 1558–1564.
[144] Nomura, T. et al. (2001) Evidence for different mechanisms of chloroquine resistance in 2 *Plasmodium* species that cause human malaria. J. Infect. Dis. 183, 1653–1661.
[145] Alam, M.T. et al. (2007) Similar trends of pyrimethamine resistance-associated mutations in *Plasmodium vivax* and *P. falciparum*. Antimicrob. Agents Chemother. 51, 857–863.
[146] Boni, M.F., Smith, D.L. and Laxminarayan, R. (2008) Benefits of using multiple first-line therapies against malaria. Proc. Natl. Acad. Sci. USA 105, 14216–14221.
[147] WHO (2009) Malaria, Countries or Areas at Risk of Transmission, WHO.
[148] WHO (2010) Global Report on Antimalarial Drug Efficacy and Drug Resistance: 2000–2010, WHO.
[149] Olliaro, P., Nevill, C., LeBras, J., Ringwald, P., Mussano, P., Garner, P. and Brasseur, P. (1996) Systematic review of amodiaquine treatment in uncomplicated malaria. Lancet 348, 1196–1201.
[150] Picot, S., Olliaro, P., de Monbrison, F., Bienvenu, A.L., Price, R.N. and Ringwald, P. (2009) A systematic review and meta-analysis of evidence for correlation between molecular markers of parasite resistance and treatment outcome in falciparum malaria. Malaria J. 8, 89.
[151] Loyevsky, M., John, C., Dickens, B., Hu, V., Miller, J.H. and Gordeuk, V.R. (1999) Chelation of iron within the erythrocytic *Plasmodium falciparum* parasite by iron chelators. Mol. Biochem. Parasitol. 101, 43–59.