Published in final edited form as:
FEBS J. 2007 September ; 274(18): 4688–4698. doi:10.1111/j.1742-4658.2007.05999.x.

Drug-resistant malaria - an insight

John E. Hyde
Manchester Interdisciplinary Biocentre, Faculty of Life Sciences, University of Manchester, UK

Abstract

Despite intensive research extending back to the 1930s, when the first synthetic antimalarial drugs made their appearance, the repertoire of clinically licensed formulations remains very limited. Moreover, widespread and increasing resistance to these drugs contributes enormously to the difficulties in controlling malaria, posing considerable intellectual, technical and humanitarian challenges. A detailed understanding of the molecular mechanisms underlying resistance to these agents is emerging that should permit new drugs to be rationally developed and older ones to be engineered to regain their efficacy. This review summarizes recent progress in analysing the causes of resistance to the major antimalarial drugs and its spread.

Keywords

antifolates; artemisinins; atovaquone; chloroquine; combination therapy; gene copy number; gene polymorphisms; *Plasmodium falciparum*

Malaria has a broad distribution throughout tropical and subtropical areas, affecting both indigenous populations and increasing numbers of travellers. The disease is caused by protozoan parasites of the genus *Plasmodium* and is transmitted via the bite of *Anopheles* mosquitoes. These parasites have a complex life cycle in both the mosquito and human hosts; in the latter, sporozoite forms injected by the mosquito during its blood meal migrate to liver cells, where, after extensive replication, merozoites are released into the bloodstream to start the cycles of erythrocyte invasion, intracellular growth and division, followed by host-cell lysis and reinvasion, which give rise to the clinical symptoms of the disease. *Plasmodium falciparum* is the most dangerous of the four species of *Plasmodium* that cause human malaria, leading to a death rate conservatively estimated as 1-2 million people per year. Such lethality stems in part from the way this particular species modifies its host erythrocyte, such that the more mature stages in this part of the life cycle adhere to endothelial surfaces and progressively block microcapillaries in major organs, such as the brain. Although malaria is still found in over 100 countries, the major burden of disease occurs in sub-Saharan Africa, where over 90% of all deaths are recorded, largely among those aged under-five, and where intense morbidity and transmission have profound consequences for public health and economic infrastructures [1].

In the continuing absence of clinically proven vaccines, preventing or treating malaria parasite infections in the human host has always depended heavily upon chemoprophylaxis and chemotherapy. Since the advent of the first synthetic antimalarials in the 1930s, only a small number of compounds has proved suitable for licensing as drugs for human use, and several of these are now greatly compromised by the inexorable spread of drug-resistant

Correspondence J. E. Hyde, Manchester Interdisciplinary Biocentre, Faculty of Life Sciences, University of Manchester, 131 Princess Street, Manchester M1 7DN, UK, Fax: +44 161 306 5201, Tel: +44 161 306 4185, E-mail: john.hyde@manchester.ac.uk.

Europe PMC Funders Author Manuscripts

parasite strains, including the cheapest formulations that are so important to Africa, namely chloroquine and pyrimethamine–sulfadoxine (a coformulated combination marketed as Fansidar®). Resistance of malaria parasites arises from several factors, including overuse of antimalarial drugs for prophylaxis, inadequate or incomplete therapeutic treatments of active infections, a high level of parasite adaptability at the genetic and metabolic levels, and a massive proliferation rate that permits selected populations to emerge relatively rapidly. With the development of sophisticated methods of molecular analysis and the ability to manipulate genes of *Plasmodium* in transfection systems, albeit with some difficulty, considerable progress has been made in understanding how *P. falciparum* has been able to subvert the chemical attacks made upon it, which in turn is suggesting new strategies for continuing the fight. Several reviews of this area with greater molecular detail and historical perspectives have been published in recent years [2-8]. This short article will focus on core mechanisms and some of the most recent developments concerning resistance to the major antimalarial drugs, as summarized in Table 1.

Chloroquine and related compounds

The 4-aminoquinoline, chloroquine, a synthetic relative of the plant-derived and quite toxic drug quinine, was for several decades the antimalarial agent of choice, as it was safe, highly effective and cheap. More recent related drugs include mefloquine (a quinoline methanol derivative), halofantrine (a phenanthrene methanol) and lumefantrine (a more complex aryl-substituted methanol), introduced in the 1980s to counter parasites that had become resistant to chloroquine and the antifolates (see below). Chloroquine resistance was first observed in Thailand in 1957 and on the Colombian-Venezuelan border in 1959. By 1988 resistance had spread to essentially all of sub-Saharan Africa and today chloroquine has lost its efficacy in all but a few areas of the world. Chloroquine was known to exert its effect by compromising the sequestration of harmful haem moieties produced as a by-product of haemoglobin digestion by the parasite, the major source of its amino acids. This process occurs in a lysosome-like acidic food (or digestive) vacuole (pH ~ 4.5-5.0), in which chloroquine accumulates to high levels in sensitive parasites, but to a much reduced degree in resistant strains. The genetic basis of this latter phenomenon was revealed by careful and extensive recombination mapping of the progeny of a genetic cross of two cloned parasite populations, one chloroquine-sensitive and the other chloroquine-resistant. The key gene, named *pfcrt* for ‘chloroquine resistance transporter’, resides on chromosome 7, encodes a 49 kDa protein with 10 predicted transmembrane domains, and exhibits mutations that showed complete linkage to the chloroquine-resistance phenotype in the 40 laboratory-adapted strains of *P. falciparum* originally examined [9]. The polymorphism in this gene documented to date is considerable, indicative of several independent origins of drug-resistant alleles. It encompasses at least 16 single, nonsynonymous nucleotide substitutions in parasites from field samples, but only one amino acid change, K76T, located in the first transmembrane domain, is found consistently in chloroquine-resistant parasites, although never on its own [6,10]. At least three other changes (and usually several more) are always seen in combination with K76T, relative to the canonical wild-type sequence, possibly compensating for unfavourable changes in the normal function of the chloroquine resistance transporter (CRT) protein induced by K76T and/or as a response to pressure from antimalarial drugs other than chloroquine that also pass through this transporter. These mutations lie mainly, but not exclusively, in the transmembrane domains, of which domains 1, 4 and 9 are thought to play particularly important roles in determining the transport characteristics of CRT with respect to different drugs [11]. cDNA versions of *pfcrt* from resistant parasites transfected into sensitive parasites bestow the resistance phenotype, and confirm the central role of codon 76 [10]. This is supported by numerous field studies conducted across the world, for example in West Africa [12] and Cambodia [13], where all

chloroquine-resistant strains examined carried the K76T, and to date, no chloroquine-resistant isolate has been found that carries the wild-type lysine at position 76.

More recent research has concentrated on classifying the CRT protein encoded by *pfcrt* and understanding its normal function, as well as the mechanism(s) by which the observed mutations might modulate chloroquine susceptibility, together with the details of drug transport across the food vacuole membrane. Bioinformatics analyses place CRT within a new subgroup of proteins belonging to a superfamily of drug and metabolite transporters (the DMT superfamily) [14,15]. Immunolocalization has confirmed that the molecule is bound into the membrane of the food vacuole and the critical K76T mutation is predicted to face the cytoplasmic side of the first transmembrane domain, where it could alter the selectivity of CRT such that chloroquine more efficiently exits the food vacuole [16,17]. Several models have been proposed to account for the observed distributions of drug between the food vacuole and cytoplasm in sensitive and resistant parasites [18]. One plausible scenario is that the positive charge of K76 normally prevents, or severely limits, the efflux of chloroquine in its diprotonated form (which predominates in the acidic vacuole), an effect that is lost after mutation to T or another nonbasic residue. However, mutations that restore a positive charge to the pore can compensate for the K76T mutation and thus reverse the chloroquine-resistance phenotype, as has been found in at least one apparently anomalous sensitive strain from South East Asia, where an S163R alteration (in the fourth transmembrane domain) is combined with K76T [16]. This ‘proton-trapping’ model, although providing an explanation for the more rapid loss of chloroquine from the vacuole of resistant parasites, does not account for the anomalously high level of drug accumulation within it that is seen in wild-type parasites. It is thus thought likely that binding of chloroquine to ferriprotoporphyrin IX (haem), once it is inside the food vacuole, will contribute significantly to the observed accumulation [19]. The level of chloroquine import into the parasite has recently been shown to depend upon the integrity of the proton gradient maintained across the vacuolar membrane [19], but the pH of the vacuole appears not to play a significant role in resistance, as both chloroquine-resistant and chloroquine-sensitive parasites have closely comparable values, when measured with a variety of molecular probes [20]. However, efflux from chloroquine-resistant lines, but not sensitive lines, appears to be energy independent, implying that the mutant form of PfCRT takes on the function of a gated channel or pore (rather than an active transporter), through which the charged chloroquine species can escape from the vacuole [19]. Attempts to exploit the above findings include studies on a range of compounds that are able to reverse chloroquine resistance, by virtue of molecular structures that are often amphipathic, with both lipophilic and positively charged regions that allow the compound to compete with chloroquine for binding to PfCRT, and hence for exit from the food vacuole [10,21].

The membrane of the food vacuole is expected to carry a number of different transporters, and another identified type is encoded by *pfmdr1* on chromosome 5, which shows similarity to the P-glycoprotein product of the human multidrug resistance gene *mdr*. The malarial protein, Pgh1 or MDR1, is a 162 kDa ABC-type transporter with 12 predicted transmembrane domains and two ATP-binding folds, thought to be involved in importing solutes into the food vacuole, including the drugs mefloquine, halofantrine and artemisinin [22]. It is now known that the mutation status of *pfmdr1* can contribute to the precise levels of chloroquine resistance, but on its own, in a *pfcrt* wild-type background, is unable to confer resistance, a conclusion supported both by transfection studies and recent field studies [18]. However, numerous reports find a reciprocal relationship between chloroquine resistance and resistance to other important quinoline- or methanol-based drugs, such as mefloquine and halofantrine, which also act on the parasite food vacuole [23]. Importantly, amplification of *pfmdr1* to copy-numbers of up to five appears to be a primary mechanism whereby parasites become resistant to these drugs, particularly in Asia [24-27], although

amino acid polymorphisms in Pgh1 can also have a significant effect [28], as can such changes in PfCRT alone [16]. It has also been proposed that putative parasite transporters other than PfCRT and Pgh1 influence the precise level of sensitivity to these drugs [29,30], but more recent work analysing the genes of nine such transporters from large numbers of parasite samples collected at a single site in South East Asia has thus far failed to support these associations [31].

Antifolates

Analysis of resistance to the antifolate drugs has been considerably more straightforward than was the case with chloroquine, as the primary molecular targets of these compounds were originally established from studies of bacteria in the 1930s and 1940s. The principal antimalarial antifolate drugs are pyrimethamine, proguanil (metabolized *in vivo* to the active form cycloguanil), the sulfonamides, such as sulfadoxine and sulfalene and the sulfone, dapsone (collectively known as the sulfa drugs). Pyrimethamine and cycloguanil target the dihydrofolate reductase (DHFR) activity of the bifunctional DHFR–thymidylate synthetase (TS) protein, while the sulfa drugs inhibit the dihydropteroate synthetase (DHPS) activity of the bifunctional hydroxymethylpterin pyrophosphokinase (HPPK)–DHPS protein. DHPS is an enzyme unique to the parasite that is used in the biosynthesis of key folate coenzymes, starting from GTP, whereas DHFR is present in both host and parasite, being essential to maintain a constant supply of fully reduced (tetra-hydro) forms of such cofactors for key one-carbon transfer reactions, including the provision of nucleotides for DNA synthesis and the metabolism of certain amino acids. The drugs that target this enzyme bind several hundred times more strongly to the parasite protein than to the human orthologue. Although pyrimethamine and proguanil were initially used alone after their introduction in the 1940/1950s, resistance arose rapidly and it was only in strongly synergistic combinations with sulfa drugs that formulations with longer term utility were produced. The main effect of such combinations is to block the parasite as it undertakes DNA replication. Despite a rapid spread of pyrimethamine–sulfadoxine resistance in South East Asia from the mid-1960s onwards, this affordable combination has been extensively used, albeit with diminishing efficacy, to combat chloroquine-resistant parasites in Africa since the early 1990s.

The genetic basis of resistance to the antifolate drugs is similar to that underlying chloroquine resistance, in that a small number of point mutations in these two target genes appear to be responsible for the major part of resistance. As well as classical drug-binding and kinetic studies on recombinant enzymes, more recent parasite transfection studies, where the resistant-type *dhfr* and *dhps* sequences are inserted into the genomes of sensitive parasites, prove directly that the mutations observed in field samples do indeed result in drug resistance in live parasites [32]. High-level pyrimethamine resistance results from the accumulation of mutations in the *dhfr* domain of *pfdhfr-ts*, principally at codons 108, 59 and 51, where the most common allelic variants encode S108N, N51I and C59R. Similarly, common patterns of mutation in *dhps* that compromise the efficacy of sulfadoxine include the codon changes A437G/K540E in Africa and A437G/A581G or A437G/K540E/A581G in South East Asia, with the last of these also often observed in areas of South America [2,5]. The most common genotypes at present found in parasites highly resistant to pyrimethamine-sulfadoxine in Africa, where such resistance is most relevant, combine triply mutated *dhfr* with doubly mutated *dhps*, a pattern that acts as a reliable marker for this phenotype [33] and a strong indicator of likely clinical outcome. In South East Asia and South America, a fourth alteration, I164L, is often found in DHFR; in combination with the above changes, this renders the mutant parasites completely resistant to achievable physiological concentrations of pyrimethamine-sulfadoxine [34]. A widespread distribution of such quadruple *dhfr* mutant parasites into Africa would be a major disaster, given that numerous countries are now using pyrimethamine-sulfadoxine as their first-line defence, and

that the most recent antifolate combination to be introduced into the field only in 2003 (chlorproguanil/dapsone, LapDap®) would also be compromised. Ominously, such parasites are now beginning to be reported from areas of East Africa [35].

An important recent milestone in malarial antifolate research was the determination of the crystal structures of DHFR-TS from both pyrimethamine-sensitive and pyrimethamine-resistant parasites, in association with anti-DHFR drugs [36]. This has boosted the search for new compounds inhibitory to this activity and increased the reliability of computer-modelled inhibitor specificities [37]. The crystal structures also help to rationalize how the resistance mutations described above alter the enzyme conformation such that drug binding is greatly reduced, while permitting sufficient processing of normal substrate [37]. As yet, no crystal structure for the HPPK–DHPS enzyme of *P. falciparum* has been reported, although homology modelling has provided insight into the role of the key resistance mutations in the DHPS domain [38].

Atovaquone and the artemisinins

Two other drug types are of particular importance as recently licensed antimalarials, although both also have a long history. The 2-hydroxynaphthoquinone derivative atovaquone stems from research into this class of mitochondrial inhibitors from the 1940s, but was only certified for use as a clinical antimalarial in combination with proguanil (as Malarone®) in 1997. Atovaquone is a structural analogue of coenzyme Q in the electron transport chain and acts to collapse the organellar membrane potential, thus arresting parasite respiration and essential pyrimidine biosynthesis. This results from inhibition of cytochrome *b* in complex III of the chain, without affecting human mitochondria, which employ a CoQ_{10} complex (i.e. one carrying 10 isoprene units on the aromatic ring), that differs from the CoQ_8 type found in the parasite. Atovaquone action is proposed to arise from its blockage of a large-scale domain movement of the iron–sulfur protein subunit that is required for electron transfer to cytochrome c_1 from ubihydroquinone bound to cytochrome *b* within complex III [39]. Where atovaquone has been used in monotherapy, resistance rapidly emerges, with point mutations in the cytochrome *b* gene, principally affecting Y268 (converted to S, N or C), associated with drastic reductions in parasite susceptibility to the drug both *in vitro* and *in vivo* [40,41]. However, in combination with proguanil, the onset of resistance is markedly retarded and Malarone has now been successfully used for some time, especially for travellers to South East Asia where multidrug-resistant parasites are common. Although the mechanism of synergy between the two components of Malarone is not yet fully understood, it appears not to be associated with the role of proguanil as an antifolate precursor, described above, but rather with its ability to sensitize the mitochondrion, possibly by blocking a secondary mechanism for maintaining its membrane potential [42]. Although apparently still completely effective as a prophylactic, some cases of resistance to Malarone treatment have been reported [43,44], not all of which were associated with mutation in the cytochrome *b* gene [45], and thus suggestive of possible alternative resistance mechanisms. Moreover, a recent study discovered the *cyt b* Y268N mutation in several *P. falciparum* isolates from Nigeria, where the population has not been exposed to atovaquone. One such isolate had an additional mutation (P266T) located at the ubiquinone reduction site of the enzyme [46]. Clearly, such observations are of concern and ongoing surveillance again will be essential to help prolong the effective life of this drug.

The artemisinins are sesquiterpene lactones that derive ultimately from the Chinese herb qinghao (*Artemisia annua*), used for centuries to treat malaria and other parasitic diseases. Their considerable potency stems in part from a highly reactive epoxide bridge across the seven-membered component of the triplering system, which is essential for antiparasitic

activity. The most effective of these compounds thus far is sodium artesunate, which can rapidly reduce parasite numbers ~ 10^4-fold in a single 48-h erythrocytic cycle. However, the (predictable) danger of using this type of drug in monotherapy, first observed in Chinese field trials in the 1970s, was emphasized by a recent study where decreased sensitivity was observed in recrudescent parasites after 7 days of treatment with artesunate alone [47]. Artesunate was originally deployed in combination with mefloquine in areas of multidrug resistance from 1994 onwards in South East Asia, where it is still used successfully, and artemisinin derivatives are now being evaluated in many other endemic areas in combinations with other antimalarials, such as lumefantrine, piperaquine, amodiaquine and sulfadoxine-pyrimethamine (so-called artemisinin combination therapy; ACT), all of which operate on longer time scales [48]. These formulations are able to eradicate the small fraction of parasites that escape the rapidly metabolized artemisinin (which has a plasma half-life of only about 4 h) and help reduce the likelihood of drug resistant forms emerging. However, there is doubt as to the effectiveness of certain of these combinations where the partner drug is already compromised [49] and there is recent evidence that resistance to the major coformulated combination of artemether-lumefantrine (Coartem®) may already be developing in parts of East Africa [50,51].

Several types of study, including immunolocalization, indicate that, upon activation by Fe^{2+}, the artemisinins inhibit the Ca^{2+}-dependent SERCA-like ATPase, PfATP6, a transporter found on membranous structures within the parasite cytoplasm [52]. However, such compounds form free radicals *in vitro* that can modify proteins and other molecules [52,53], which could also play a role *in vivo*. Studies in a yeast model suggested that disruption of the membrane potential of the mitochondrion might be an important factor [54], although localization of artemisinins to the mitochondria of *P. falciparum* has not been demonstrated and the relevance of these observations to the parasite system is still unclear [53]. More pertinent to the PfATP6 hypothesis, it has recently been shown in a heterologous (*Xenopus* oocyte) system that mutations in the key residue L263 close to the inhibitor binding site of this enzyme can abolish sensitivity to artemisinins. Disturbingly, field isolates that show reduced susceptibility to artemether associated with a S769N mutation in PfATP6 (but not with *pfcrt* or *pfmdr1* genotypes) have been found in areas of French Guiana where uncontrolled use of artemisinin derivatives has occurred [55]. This serine residue is thought to be involved in a conformational change around a conserved hinge region of the molecule. These studies support the hypothesis that PfATP6, although possibly not the sole target, is a key player, as well as suggesting how artemisinin derivatives may be further developed in future [56,57]. Stable resistance to artemisinin and artesunate has also been induced in laboratory lines of the rodent parasite *Plasmodium chabaudi*, but no mutations or copy number changes were apparent in any of the genes that have been potentially implicated in the resistance of *P. falciparum* and other malarial species, including the homologue of *pfatp6* [58]. This may reflect genuine differences between the distantly related human and rodent species of *Plasmodium*, which may not be relevant to *P. falciparum*, or, perhaps more likely, a warning that resistance in the latter may also eventually arise in the field by as yet unknown genetic alterations.

Concluding remarks

In general, microorganisms can employ a range of mechanisms to overcome drug challenge, in addition to structural modification of the target protein. These include amplification of the gene encoding the target, or its upregulation during transcription or translation, or by compromising the drug itself by inactivation or sequestration. However, to date, the principal (but not sole) strategy of *P. falciparum* appears to result in one or a small number of point mutations in genes encoding the key proteins. One lesson that is emerging is that certain of these mutations at the amino acid level directly affect drug binding or transport,

whereas others are almost certainly compensatory, to permit the parasite to continue processing native substrates at levels that, although possibly suboptimal, are still compatible with viability. This raises the attractive possibility of administering simultaneously drugs that target the same molecule, but which by subtle structural variation, would select on their own mutually incompatible combinations of mutations, as proposed nearly a decade ago for the antifolates [59]. Proof of principle of such an idea has now been demonstrated for the case of *Plasmodium vivax*, where the mutations that confer high-level resistance to pyrimethamine cause the DHFR-TS enzyme to become up to 10-fold more sensitive to the powerful (nonclinical) antifolate, WR99210, compared with the wild-type [60]. Studies are currently underway to establish whether this phenomenon can be extended to *P. falciparum*, as seems likely from earlier work on pyrimethamine and WR99210 selection on randomly mutated *pfdhfr* libraries [61]. Similarly, the observation that primaquine, used for decades to clear the liver of the long-lived hypnozoite form of the parasite peculiar to *P. vivax* and *Plasmodium ovale*, can act as a chloroquine resistance reversal agent in *P. falciparum*, leads to the suggestion of combining these two extremely cheap drugs [62], and there is also evidence that Coartem and amodiaquine exert opposing selective pressures on the *pfmdr1* gene [63]. Such approaches complement the strategy of deploying drug combinations against two (or more) unrelated targets, described above.

Although considerable progress has now been made in detailing underlying mechanisms, it would not be surprising if other, as yet unidentified, factors contribute to the precise levels of susceptibility to the various antimalarial drugs. For example, there is a recent suggestion that variation in copy number of the gene encoding GTP cyclohydrolase I, at the entry point of the folate biosynthetic pathway, may modulate levels of antifolate resistance in *P. falciparum* [64], although varying expression levels of *pfcrt* appear to have little influence on susceptibility to chloroquine *in vitro* [13]. As described above, copy number has been established as a major factor in the relationship of the *pfmdr1* gene to mefloquine resistance. With regard to the host, their history of exposure to the parasite and hence immune status will influence the clinical outcome of drug treatment, and in the case of the antifolates, nutritional status can play a significant role, as higher levels of blood folate reduce the efficacy of pyrimethamine-sulfadoxine [65]. However, the seeming general predominance of point mutations in underlying resistance in the parasite, now well established for a number of key genes, at least permits relatively straightforward epidemiological surveys based on PCR, which can be multiplexed to provide information on several genes simultaneously [66,67], not only from patient blood samples, but from mosquito populations as well [68]. Importantly, such surveys can also include longitudinal studies of samples stored long ago, before key genes and their allelic variants were identified. For example, it has now been shown that, after chloroquine was withdrawn from use in Malawi in 1993, prevalence of the resistant form of *pfcrt* decreased from about 85% in 1992 to an undetectable level in 2001 [69]. A similar increase in frequency of the wild-type allele has been observed for the *dhfr* domain of the *pfdhfr-ts* gene following reduced usage of pyrimethamine–sulfadoxine in an area of Tanzania, thanks to the deployment of bed-net protection from mosquito bites [70]. In the case of atovaquone resistance, mutations in the *cyt b* gene also appear to result in loss of fitness [71], as do those in the *pfmdr1* gene [72]. However, parasites mutant in *dhfr* are still prevalent in parts of South East Asia and elsewhere, despite low usage of antifolates in these areas for prolonged periods, suggesting that in some contexts, the mutant parasites retain a small but significant selective advantage [73]. Such studies are highly valuable when considering changes in a given drug regime at a national or more local level [74]. Related to this are the analyses of variable DNA sequences flanking the coding regions of the target genes from large numbers of geographically diverse field samples, which also provide considerable insight into the evolution and spread of drug-resistant strains. Unexpectedly perhaps, the picture that has emerged here, both for the antifolates and chloroquine [75,76], is consistent with the widespread migration of a relatively small

number of rare ancestral mutant alleles across countries and continents, rather than the frequent, independent genesis of mutant types in numerous different locations where drug pressure is strong. However, some data on *pfdhfr* from western Kenya that are more compatible with the latter scenario have also been reported [35], as has a distinct lineage of antifolate-resistant strains originating in the Melanesian islands [77].

The complete genome sequences of several *Plasmodium* species, including *P. falciparum*, are now known, and discoveries in this fertile postgenomic era suggest a plethora of new areas to explore, exemplified by the broad spectrum of potential targets that can be envisaged, for example, in just one area of metabolism, that of purine and pyrimidine biochemistry [78]. Other studies, too numerous to detail here, envisage deployment of entirely new classes of drugs, targeted, for example, to recently discovered metabolic pathways in essential organelles of the parasite, the apicoplast [79] and the mitochondrion [80,81]. However, the translation of fundamental research into clinically licensed drugs that operate in new ways or can overcome resistant forms of the parasite is a formidable challenge, and careful assessment of the most promising of these targets is required to validate them as *bona fide* candidates, before the labour- and cost-intensive commitment to long-term development. The latter of course entails considerable hurdles in the many steps between identification of a seemingly attractive enzyme or transporter target and the eventual formulation of a safe and effective medicine, where problems of solubility, effective delivery, toxicity to the host and other potential pitfalls must first be tackled. Better though to be spoilt for choice, rather than wondering where the next target is coming from, as was the case not so many years ago.

Acknowledgments

The restricted number of references permitted for this article has meant omitting many citations to primary literature, for which I tender apologies to the affected authors. Relevant work carried out in my laboratory was funded principally by grants 056845 and 073896 from the Wellcome Trust, UK.

Abbreviations

CRT	chloroquine resistance transporter
DHFR	dihydrofolate reductase
DHPS	dihydropteroate synthetase
HPPK	hydroxymethylpterin pyrophosphokinase
TS	thymidylate synthetase

References

1. Suh KN, Kain KC, Keystone JS. Malaria. Can Med Assoc J. 2004; 170:1693–1702. [PubMed: 15159369]
2. Hyde JE. Mechanisms of resistance of *Plasmodium falciparum* to antimalarial drugs. Microbes Infect. 2002; 4:165–174. [PubMed: 11880048]
3. Talisuna AO, Bloland P, D'Alessandro U. History, dynamics, and public health importance of malaria parasite resistance. Clin Microbiol Rev. 2004; 17:235–254. [PubMed: 14726463]
4. Arav-Boger R, Shapiro TA. Molecular mechanisms of resistance in antimalarial chemotherapy: the unmet challenge. Annu Rev Pharmacol Toxicol. 2005; 45:565–585. [PubMed: 15822189]
5. Gregson A, Plowe CV. Mechanisms of resistance of malaria parasites to antifolates. Pharmacol Rev. 2005; 57:117–145. [PubMed: 15734729]

6. Cooper RA, Hartwig CL, Ferdig MT. pfcrt is more than the *Plasmodium falciparum* chloroquine resistance gene: a functional and evolutionary perspective. Acta Trop. 2005; 94:170–180. [PubMed: 15866507]
7. Nzila A. The past, present and future of antifolates in the treatment of *Plasmodium falciparum* infection. J Antimicrob Chemother. 2006; 57:1043–1054. [PubMed: 16617066]
8. Woodrow CJ, Krishna S. Antimalarial drugs: recent advances in molecular determinants of resistance and their clinical significance. Cell Mol Life Sci. 2006; 63:1586–1596. [PubMed: 16699808]
9. Fidock DA, Nomura T, Talley AK, Cooper RA, Dzekunov SM, Ferdig MT, Ursos LMB, bir Singh Sidhu A, Naude B, Deitsch KW, et al. Mutations in the *P. falciparum* digestive vacuole transmembrane protein PfCRT and evidence for their role in chloroquine resistance. Mol Cell. 2000; 6:861–871. TE. [PubMed: 11090624]
10. Bray PG, Martin RE, Tilley L, Ward SA, Kirk K, Fidock DA. Defining the role of PfCRT in *Plasmodium falciparum* chloroquine resistance. Mol Microbiol. 2005; 56:323–333. [PubMed: 15813727]
11. Cooper RA, Lane KD, Deng BB, Mu JB, Patel JJ, Wellems TE, Su XZ, Ferdig MT. Mutations in transmembrane domains 1, 4 and 9 of the *Plasmodium falciparum* chloroquine resistance transporter alter susceptibility to chloroquine, quinine and quinidine. Mol Microbiol. 2007; 63:270–282. [PubMed: 17163969]
12. Djimde A, Doumbo OK, Cortese JF, Kayentao K, Doumbo S, Diourte Y, Dicko A, Su XZ, Nomura T, Fidock DA, et al. A molecular marker for chloroquine-resistant falciparum malaria. N Engl J Med. 2001; 344:257–263. [PubMed: 11172152]
13. Durrand V, Berry A, Sem R, Glaziou P, Beaudou J, Fandeur T. Variations in the sequence and expression of the *Plasmodium falciparum* chloroquine resistance transporter (Pfcrt) and their relationship to chloroquine resistance *in vitro*. Mol Biochem Parasitol. 2004; 136:273–285. [PubMed: 15478806]
14. Tran CV, Saier MH. The principal chloroquine resistance protein of *Plasmodium falciparum* is a member of the drug/metabolite transporter superfamily. Microbiology (UK). 2004; 150:1–3.
15. Martin RE, Kirk K. The malaria parasite's chloroquine resistance transporter is a member of the drug/metabolite transporter superfamily. Mol Biol Evol. 2004; 21:1938–1949. [PubMed: 15240840]
16. Johnson DJ, Fidock DA, Mungthin M, Lakshmanan V, Sidhu ABS, Bray PG, Ward SA. Evidence for a central role for PfCRT in conferring *Plasmodium falciparum* resistance to diverse antimalarial agents. Mol Cell. 2004; 15:867–877. [PubMed: 15383277]
17. Lakshmanan V, Bray PG, Verdier-Pinard D, Johnson DJ, Horrocks P, Muhle RA, Alakpa GE, Hughes RH, Ward SA, Krogstad DJ, et al. A critical role for PfCRT K76T in *Plasmodium falciparum* verapamil-reversible chloroquine resistance. EMBO J. 2005; 24:2294–2305. [PubMed: 15944738]
18. Valderramos SG, Fidock DA. Transporters involved in resistance to antimalarial drugs. Trends Pharmacol Sci. 2006; 27:594–601. [PubMed: 16996622]
19. Bray PG, Mungthin M, Hastings IM, Biagini GA, Saidu DK, Lakshmanan V, Johnson DJ, Hughes RH, Stocks PA, O'Neill PM, et al. PfCRT and the trans-vacuolar proton electrochemical gradient: regulating the access of chloroquine to ferriprotoporphyrin IX. Mol Microbiol. 2006; 62:238–251. [PubMed: 16956382]
20. Hayward R, Saliba KJ, Kirk K. The pH of the digestive vacuole of *Plasmodium falciparum* is not associated with chloroquine resistance. J Cell Sci. 2006; 119:1016–1025. [PubMed: 16492710]
21. Henry M, Alibert S, Orlandi-Pradines E, Bogreau H, Fusai T, Rogier C, Barbe J, Pradines B. Chloroquine resistance reversal agents as promising antimalarial drugs. Current Drug Targets. 2006; 7:935–948. [PubMed: 16918322]
22. Rohrbach P, Sanchez CP, Hayton K, Friedrich O, Patel J, Sidhu ABS, Ferdig MT, Fidock DA, Lanzer M. Genetic linkage of *pfmdr1* with food vacuolar solute import in *Plasmodium falciparum*. EMBO J. 2006; 25:3000–3011. [PubMed: 16794577]
23. Duraisingh MT, Cowman AF. Contribution of the *pfmdr1* gene to antimalarial drug resistance. Acta Trop. 2005; 94:181–190. [PubMed: 15876420]

24. Price RN, Uhlemann AC, Brockman A, McGready R, Ashley E, Phaipun L, Patel R, Laing K, Looareesuwan S, White NJ, et al. Mefloquine resistance in *Plasmodium falciparum and increased pfmdr1* gene copy number. Lancet. 2004; 364:438–447. [PubMed: 15288742]
25. Sidhu ABS, Uhlemann AC, Valderramos SG, Valderramos JC, Krishna S, Fidock DA. Decreasing *pfmdr1* copy number in *Plasmodium falciparum* malaria heightens susceptibility to mefloquine, lumefantrine, halofantrine, quinine, and artemisinin. J Infect Dis. 2006; 194:528–535. [PubMed: 16845638]
26. Alker AP, Lim P, Sem R, Shah NK, Yi P, Bouth DM, Tsuyuoka R, Maguire JD, Fandeur T, Ariey F, et al. *PFMDR1* and *in vivo* resistance to artesunate-mefloquine in falciparum malaria on the Cambodian-Thai border. Am J Trop Med Hyg. 2007; 76:641–647. [PubMed: 17426163]
27. Uhlemann AC, McGready R, Ashley EA, Brockman A, Singhasivanon P, Krishna S, White NJ, Nosten F, Price RN. Intrahost selection of *Plasmodium falciparum pfmdr1* alleles after antimalarial treatment on the northwestern border of Thailand. J Infect Dis. 2007; 195:134–141. [PubMed: 17152017]
28. Sidhu ABS, Valderramos SG, Fidock DA. pfmdr1 mutations contribute to quinine resistance and enhance mefloquine and artemisinin sensitivity in Plasmodium falciparum. Mol Microbiol. 2005; 57:913–926. [PubMed: 16091034]
29. Mu JB, Ferdig MT, Feng XR, Joy DA, Duan JH, Furuya T, Subramanian G, Aravind L, Cooper RA, Wootton JC, et al. Multiple transporters associated with malaria parasite responses to chloroquine and quinine. Mol Microbiol. 2003; 49:977–989. [PubMed: 12890022]
30. Ferdig MT, Cooper RA, Mu JB, Deng BB, Joy DA, Su XZ, Wellems TE. Dissecting the loci of lowlevel quinine resistance in malaria parasites. Mol Microbiol. 2004; 52:985–997. [PubMed: 15130119]
31. Anderson TJC, Nair S, Qin H, Singlam S, Brockman A, Paiphun L, Nosten F. Are transporter genes other than the chloroquine resistance locus (*pfcrt*) and multidrug resistance gene (*pfmdr*) associated with antimalarial drug resistance? Antimicrob Agents Chemother. 2005; 49:2180–2188. [PubMed: 15917511]
32. Crabb BS. Transfection technology and the study of drug resistance in the malaria parasite *Plasmodium falciparum*. Drug Resist Update. 2002; 5:126–130.
33. Kublin JG, Dzinjalamala FK, Kamwendo DD, Malkin EM, Cortese JF, Martino LM, Mukadam RAG, Rogerson SJ, Lescano AG, Molyneux ME, et al. Molecular markers for failure of sulfadoxine-pyrimethamine and chlorproguanil-dapsone treatment of *Plasmodium falciparum* malaria. J Infect Dis. 2002; 185:380–388. [PubMed: 11807721]
34. Sibley CH, Hyde JE, Sims PFG, Plowe CV, Kublin JG, Mberu EK, Cowman AF, Winstanley PA, Watkins WM, Nzila AM. Pyrimethamine-sulfadoxine resistance in *Plasmodium falciparum*: what next? Trends Parasitol. 2001; 17:582–588. [PubMed: 11756042]
35. McCollum AM, Poe AC, Hamel M, Huber C, Zhou ZY, Shi YP, Ouma P, Vulule J, Bloland P, Slutsker L, et al. Antifolate resistance in *Plasmodium falciparum*: multiple origins and identification of novel *dhfr* alleles. J Infect Dis. 2006; 194:189–197. [PubMed: 16779725]
36. Yuvaniyama J, Chitnumsub P, Kamchonwongpaisan S, Vanichtanankul J, Sirawaraporn W, Taylor P, Walkinshaw MD, Yuthavong Y. Insights into antifolate resistance from malarial DHFR-TS structures. Nat Struct Biol. 2003; 10:357–365. [PubMed: 12704428]
37. Yuthavong Y, Yuvaniyama J, Chitnumsub P, Vanichtanankul J, Chusacultanachai S, Tarnchompoo B, Vilaivan T, Kamchonwongpaisan S. Malarial (*Plasmodium falciparum*) dihydrofolate reductase-thymidylate synthase: structural basis for antifolate resistance and development of effective inhibitors. Parasitology. 2005; 130:249–259. [PubMed: 15796007]
38. de Beer TAP, Louw AI, Joubert F. Elucidation of sulfadoxine resistance with structural models of the bifunctional *Plasmodium falciparum* dihydropterin pyrophosphokinase-dihydropteroate synthase. Bioorganic Med Chem. 2006; 14:4433–4443.
39. Mather MW, Darrouzet E, Valkova-Valchanova M, Cooley JW, McIntosh MT, Daldal F, Vaidya AB. Uncovering the molecular mode of action of the antimalarial drug atovaquone using a bacterial system. J Biol Chem. 2005; 280:27458–27465. [PubMed: 15917236]

40. Korsinczky M, Chen NH, Kotecka B, Saul A, Rieckmann K, Cheng Q. Mutations in *Plasmodium falciparum* cytochrome *b* that are associated with atovaquone resistance are located at a putative drug-binding site. Antimicrob Agents Chemother. 2000; 44:2100–2108. [PubMed: 10898682]
41. Berry A, Senescau A, Lelievre J, Benoit-Vical F, Fabre R, Marchou B, Magnaval JF. Prevalence of *Plasmodium falciparum* cytochrome *b* gene mutations in isolates imported from Africa, and implications for atovaquone resistance. Trans Roy Soc Trop Med Hyg. 2006; 100:986–988. [PubMed: 16690094]
42. Painter HJ, Morrisey JM, Mather MW, Vaidya AB. Specific role of mitochondrial electron transport in blood-stage *Plasmodium falciparum*. Nature. 2007; 446:88–91. [PubMed: 17330044]
43. Kuhn S, Gill MJ, Kain KC. Emergence of atovaquone-proguanil resistance during treatment of *Plasmodium falciparum* malaria acquired by a non-immune North American traveller to West Africa. Am J Trop Med Hyg. 2005; 72:407–409. [PubMed: 15827276]
44. Krudsood S, Patel SN, Tangpukdee N, Thanachartwet W, Leowattana W, Pornpininworakij K, Boggild AK, Looareesuwan S, Kain KC. Efficacy of atovaquone-proguanil for treatment of acute multidrug-resistant *Plasmodium falciparum* malaria in Thailand. Am J Trop Med Hyg. 2007; 76:655–658. [PubMed: 17426165]
45. Wichmann O, Muehlen M, Gruss H, Mockenhaupt FP, Suttorp N, Jelinek T. Malarone treatment failure not associated with previously described mutations in the cytochrome *b* gene. Malaria J. 2004 doi:10.1186/1475-2875-3-14.
46. Happi CT, Gbotosho GO, Folarin OA, Milner D, Sarr O, Sowunmi A, Kyle DE, Milhous WK, Wirth DF, Oduola AMJ. Confirmation of emergence of mutations associated with atovaquone-proguanil resistance in unexposed *Plasmodium falciparum* isolates from Africa. Malaria J. 2006 doi:10.1186/1475-2875-5-82.
47. Menard D, Matsika-Claquin MD, Djalle D, Yapou F, Manirakiza A, Dolmazon V, Sarda J, Talarmin A. Association of failures of seven-day courses of artesunate in a non-immune population in Bangui, Central African Republic with decreased sensitivity of *Plasmodium falciparum*. Am J Trop Med Hyg. 2005; 73:616–621. [PubMed: 16172492]
48. Olliaro PL, Taylor WRJ. Antimalarial compounds: from bench to bedside. J Exp Biol. 2003; 206:3753–3759. [PubMed: 14506210]
49. Duffy PE, Mutabingwa TK. Artemisinin combination therapies. Lancet. 2006; 367:2037–2039. [PubMed: 16798368]
50. Sisowath C, Stromberg J, Martensson A, Msellem M, Obondo C, Bjorkman A, Gil JP. *In vivo* selection of *Plasmodium falciparum pfmdr1*, 86N coding alleles by artemether-lumefantrine (Coartem). J Infect Dis. 2005; 191:1014–1017. [PubMed: 15717281]
51. Dokomajilar C, Nsobya SL, Greenhouse B, Rosenthal PJ, Dorsey G. Selection of *Plasmodium falciparum pfmdr1* alleles following therapy with artemether-lumefantrine in an area of Uganda where malaria is highly endemic. Antimicrob Agents Chemother. 2006; 50:1893–1895. [PubMed: 16641472]
52. Eckstein-Ludwig U, Webb RJ, van Goethem IDA, East JM, Lee AG, Kimura M, O'Neill PM, Bray PG, Ward SA, Krishna S. Artemisinins target the SERCA of *Plasmodium falciparum*. Nature. 2003; 424:957–961. [PubMed: 12931192]
53. Krishna S, Woodrow CJ, Staines HM, Haynes RK, Mercereau-Puijalon O. Re-evaluation of how artemisinins work in light of emerging evidence of *in vitro* resistance. Trends Mol Med. 2006; 12:200–205. [PubMed: 16616639]
54. Li W, Mo WK, Shen D, Sun LB, Wang J, Lu S, Gitschier JM, Zhou B. Yeast model uncovers dual roles of mitochondria in the action of arternisinin. PLoS Genet. 2005; 1:329–334.
55. Jambou R, Legrand E, Niang M, Khim N, Lim P, Volney B, Ekala MT, Bouchier C, Esterre P, Fandeur T, et al. Resistance of *Plasmodium falciparum* field isolates to *in vitro* artemether and point mutations of the SERCA-type PfATPase6. Lancet. 2005; 366:1960–1963. [PubMed: 16325698]
56. Krishna S, Uhlemann AC, Haynes RK. Artemisinins: mechanisms of action and potential for resistance. Drug Resist Update. 2004; 7:233–244.

57. Uhlemann AC, Cameron A, Eckstein-Ludwig U, Fischbarg J, Iserovich P, Zuniga FA, East M, Lee A, Brady L, Haynes RKS, et al. A single amino acid residue can determine the sensitivity of SERCAs to artemisinins. Nat Struct Mol Biol. 2005; 12:628–629. [PubMed: 15937493]
58. Afonso A, Hunt P, Cheesman S, Alves AC, Cunha CV, do Rosario V, Cravo P. Malaria parasites can develop stable resistance to artemisinin but lack mutations in candidate genes *atp6* (encoding the sarcoplasmic and endoplasmic reticulum Ca^{2+} ATPase), *tctp*, *mdr1*, and *cg10*. Antimicrob Agents Chemother. 2006; 50:480–489. [PubMed: 16436700]
59. Sirawaraporn W, Sathitkul T, Sirawaraporn R, Yuthavong Y, Santi DV. Antifolate-resistant mutants of *Plasmodium falciparum* dihydrofolate reductase. Proc Natl Acad Sci USA. 1997; 94:1124–1129. [PubMed: 9037017]
60. Hastings MD, Sibley CH. Pyrimethamine and WR99210 exert opposing selection on dihydrofolate reductase from *Plasmodium vivax*. Proc Natl Acad Sci USA. 2002; 99:13137–13141. [PubMed: 12198181]
61. Chusacultanachai S, Thiensathit P, Tarnchompoo B, Sirawaraporn W, Yuthavong Y. Novel antifolate resistant mutations of *Plasmodium falciparum* dihydrofolate reductase selected in *Escherichia coli*. Mol Biochem Parasitol. 2002; 120:61–72. [PubMed: 11849706]
62. Egan TJ. Chloroquine and primaquine: combining old drugs as a new weapon against falciparum malaria? Trends Parasitol. 2006; 22:235–237. [PubMed: 16580880]
63. Humphreys GS, Merinopoulos I, Ahmed J, Whitty CJM, Mutabingwa TK, Sutherland CJ, Hallett RL. Amodiaquine and artemether-lumefantrine select distinct alleles of the *Plasmodium falciparum mdr1* gene in Tanzanian children treated for uncomplicated malaria. Antimicrob Agents Chemother. 2007; 51:991–997. [PubMed: 17194834]
64. Kidgell C, Volkman SK, Daily J, Borevitz JO, Plouffe D, Zhou YY, Johnson JR, Le Roch KG, Sarr O, Ndir O, et al. A systematic map of genetic variation in *Plasmodium falciparum*. PLoS Pathogens. 2006; 2:562–577.
65. Dzinjalamala FK, Macheso A, Kublin JG, Taylor TE, Barnes KI, Molyneux ME, Plowe CV, Smith PJ. Blood folate concentrations and *in vivo* sulfadoxine-pyrimethamine failure in Malawian children with uncomplicated *Plasmodium falciparum* malaria. Am J Trop Med Hyg. 2005; 72:267–272. [PubMed: 15772319]
66. Veiga MI, Ferreira PE, Bjorkman A, Gil JP. Multiplex PCR-RFLP methods for *pfcrt*, *pfmdr1* and *pfdhfr* mutations in *Plasmodium falciparum*. Mol Cell Probes. 2006; 20:100–104. [PubMed: 16460912]
67. Carnevale EP, Kouri D, Dare JT, McNamara DT, Mueller I, Zimmerman PA. A multiplex ligase detection reaction-fluorescent microsphere assay for simultaneous detection of single nucleotide polymorphisms associated with *Plasmodium falciparum* drug resistance. J Clin Microbiol. 2007; 45:752–761. [PubMed: 17121999]
68. Temu EA, Kimani I, Tuno N, Kawada H, Minjas JN, Takagi M. Monitoring chloroquine resistance using *Plasmodium falciparum* parasites isolated from wild mosquitoes in Tanzania. Am J Trop Med Hyg. 2006; 75:1182–1187. [PubMed: 17172390]
69. Laufer MK, Thesing PC, Eddington ND, Masonga R, Dzinjalamala FK, Takala SL, Taylor TE, Plowe CV. Return of chloroquine antimalarial efficacy in Malawi. N Engl J Med. 2006; 355:1959–1966. [PubMed: 17093247]
70. Alifrangis M, Lemnge MM, Ronn AM, Segeja MD, Magesa SM, Khalil IF, Bygbjerg IC. Increasing prevalence of wildtypes in the dihydrofolate reductase gene of *Plasmodium falciparum* in an area with high levels of sulfadoxine/pyrimethamine resistance after introduction of treated bed nets. Am J Trop Med Hyg. 2003; 69:238–243. [PubMed: 14628937]
71. Peters JM, Chen NH, Gatton M, Korsinczky M, Fowler EV, Manzetti S, Saul A, Cheng Q. Mutations in cytochrome *b* resulting in atovaquone resistance are associated with loss of fitness in *Plasmodium falciparum*. Antimicrob Agents Chemother. 2002; 46:2435–2441. [PubMed: 12121915]
72. Hayward R, Saliba KJ, Kirk K. *pfmdr1* mutations associated with chloroquine resistance incur a fitness cost in *Plasmodium falciparum*. Mol Microbiol. 2005; 55:1285–1295. [PubMed: 15686571]
73. Marks F, Evans J, Meyer CG, Browne EN, Flessner C, von Kalckreuth V, Eggelte TA, Horstmann RD, May J. High prevalence of markers for sulfadoxine and pyrimethamine resistance in

Plasmodium falciparum in the absence of drug pressure in the Ashanti Region of Ghana. Antimicrob Agents Chemother. 2005; 49:1101–1105. [PubMed: 15728909]

74. Plowe CV. Antimalarial drug resistance in Africa: strategies for monitoring and deterrence. Curr Top Microbiol Immunol. 2005; 295:55–79. [PubMed: 16265887]
75. Anderson TJC, Roper C. The origins and spread of antimalarial drug resistance: lessons for policy makers. Acta Trop. 2005; 94:269–280. [PubMed: 15878153]
76. Ariey F, Fandeur T, Durand R, Randrianarivelojosia M, Jambou R, Legrand E, Ekala MT, Bouchier C, Cojean S, Duchemin JB, et al. Invasion of Africa by a single *pfcrt* allele of South East Asian type. Malaria J. 2006 doi:10.1186/1475-2875-5-34.
77. Mita T, Tanabe K, Takahashi N, Tsukahara T, Eto H, Dysoley L, Ohmae H, Kita K, Krudsood S, Looareesuwan S, et al. Independent evolution of pyrimethamine resistance in *Plasmodium falciparum* isolates in Melanesia. Antimicrob Agents Chemother. 2007; 51:1071–1077. [PubMed: 17210777]
78. Hyde JE. Targeting purine and pyrimidine metabolism in human apicomplexan parasites. Curr Drug Targets. 2007; 8:31–47. [PubMed: 17266529]
79. Ralph SA, van Dooren GG, Waller RF, Crawford MJ, Fraunholz MJ, Foth BJ, Tonkin CJ, Roos DS, McFadden GI. Metabolic maps and functions of the *Plasmodium falciparum* apicoplast. Nat Rev Microbiol. 2004; 2:203–216. [PubMed: 15083156]
80. van Dooren GG, Stimmler LM, McFadden GI. Metabolic maps and functions of the *Plasmodium* mitochondrion. FEMS Microbiol Rev. 2006; 30:596–630. [PubMed: 16774588]
81. Mather MW, Henry KW, Vaidya AB. Mitochondrial drug targets in apicomplexan parasites. Curr Drug Targets. 2007; 8:49–60. [PubMed: 17266530]

Europe PMC Funders Author Manuscripts

Europe PMC Funders Author Manuscripts

Table 1

P. falciparum proteins with a proven role in resistance to clinical antimalarial drugs

Protein	Function	Location	Principal drugs affected[a]	Comments	Ref[b]
CRT	Transporter	Membrane of food vacuole	Chloroquine Mefloquine, halofantrine, lumefantrine, artemisinins, quinine	Major determinant Minor determinant	[3,6,8,18]
Pgh1 (P-glycoprotein homologue 1) or MDR1 (multidrug resistance 1)	Transporter	Membrane of food vacuole	Mefloquine, halofantrine, lumefantrine, quinine (possibly)	Major determinant	[8,18,23]
			Minor determinant	Chloroquine, artemisinins	[8,18,23]
DHPS	Folate pathway enzyme	Cytoplasm (principally)	Sulfadoxine, dapsone	DHPS and DHFR targeted simultaneously in synergistic combinations of antifolates	[2,3,5]
DHFR	Folate pathway enzyme	Cytoplasm (principally)	Pyrimethamine, proguanil, chlorproguanil	DHPS and DHFR targeted simultaneously in synergistic combinations of antifolates	[2,3,5]
Cytochrome *b*	Subunit of complex III (cytochrome bc_1 complex) electron transport chain	Mitochondrion	Atovaquone		[40,46]
ATP6 (sarco/endoplasmic reticulum calcium-dependent ATPase [SERCA] orthologue)	Membrane-bound Ca^{2+} -transporting ATPase	Membranous structures within cytoplasm	Artemisinins	Likely major determinant	[53,55]

[a] Only drugs discussed in the review are listed.

[b] References (mainly reviews) that describe the polymorphisms affecting each protein in detail.