

# Medical need, scientific opportunity and the drive for antimalarial drugs

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Continued and sustainable improvements in antimalarial medicines through focused research and development are essential for the world's future ability to treat and control malaria. Unfortunately, malaria is a disease of poverty, and despite a wealth of scientific knowledge there is insufficient market incentive to generate the competitive, business-driven industrial antimalarial drug research and development that is normally needed to deliver new products. Mechanisms of partnering with industry have been established to overcome this obstacle and to open up and build on scientific opportunities for improved chemotherapy in the future.

**W**hen contemplating any review of chemotherapy it is important to recognize that there is no such thing as a perfect drug. All pharmaceutical agents have their liabilities as well as their benefits. The adverse effects of some drugs, such as thalidomide<sup>1</sup>, have had a big impact on the types of study that are now required to obtain regulatory approval to market drugs. Regulatory studies place great emphasis on drug safety and on quality assurance of product, as well as on efficacy. 'Pharmacovigilance' — the continued monitoring and surveillance of drug safety after marketing authorization has been granted — is also essential to good pharmaceutical practice.

For infectious diseases, an additional liability and a chief motivation for continued innovation is the development of drug resistance. Malaria had been sheltered for many years from the dangers of resistance because of the outstanding properties of chloroquine and the slow speed at which resistance developed to this drug<sup>2</sup>. But the final arrival of resistance to chloroquine on a global scale has exposed the ease with which resistance may develop to other drugs such as the antifolates<sup>3,4</sup>. Figure 1 illustrates the dilemma that now faces malaria control authorities,

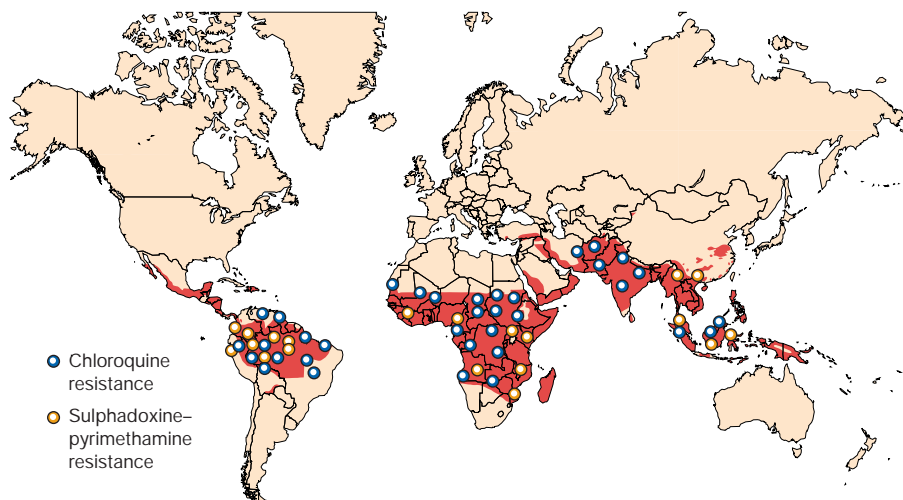
with global resistance prevailing against the two most widely used antimalarial drugs, chloroquine and the antifolate sulphadoxine/pyrimethamine. The challenge ahead lies in determining the best alternative therapies available for use now, the best prospects for drug development, regulatory approval and use in the short term, and the establishment of mechanisms and projects to ensure that improved drugs are sustainably discovered and developed into the future.

For brevity, I review primarily work carried out on the most significant of the four species of malaria parasite that infect humans, *Plasmodium falciparum*, and do not cover work on *Plasmodium vivax*, *Plasmodium ovale* or *Plasmodium malariae* in detail. I also focus on drugs and research directed against the erythrocytic stage of the parasite life cycle — the stage that gives rise to the clinical symptoms of the disease. I do not address the liver stage of infection.

## Benefits and liabilities of existing antimalarial drugs

A good starting point for anybody interested in the detailed clinical properties of existing antimalarial drugs and their impact on antimalarial drug policy is a series of reports by the WHO (World Health Organization) on the use of antimalarial drugs<sup>5-7</sup>. The molecular aspects of malaria

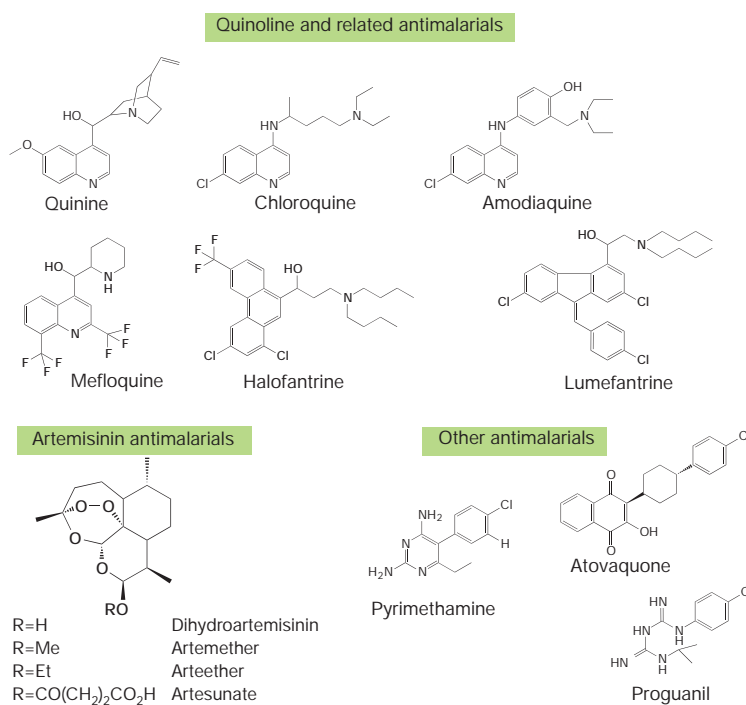
**Figure 1** Global status of resistance to chloroquine and sulphadoxine/pyrimethamine, the two most widely used antimalarial drugs. Data are from the WHO.



## Box 1

## Overview of antimalarial drugs

Drug	Main limitations
Chloroquine Quinine Amodiaquine Mefloquine Halofantrine	Resistance Compliance/safety/resistance Safety/resistance (Safety)/resistance/(cost) Safety/resistance/cost
Artemisinins (artemether, arteether, artesunate)	Compliance/(safety)/(GMP)/ (cost)
Sulphadoxine- pyrimethamine	Resistance
Atovaquone- proguanil	Resistance potential/cost
Lumefantrine- artemether	(Compliance)/resistance potential/(cost)



Drugs that are currently in use as antimalarials have many attributes, but also possess certain liabilities that might be improved by further drug discovery and development. Liabilities placed in brackets refer to issues that are less serious for the drugs in question than those liabilities not placed in brackets. The structures of the main antimalarial drugs are provided for reference.

chemotherapy are well covered by a collection of articles in ref. 8. The principal antimalarial drugs in use are listed in Box 1.

### Quinolines

Quinoline antimalarials and related aryl alcohols owe their origins to quinine — an active ingredient of *Cinchona* bark, which was first imported into Europe from Peru for antimalarial use in the seventeenth century<sup>9</sup>. Quinine has liabilities associated with toxicity (such as tinnitus) and, because it requires thrice daily administration over 7 days, this can result in poor compliance. The dependence on raw material for its extraction and the opportunities presented by its structural elucidation led to the development of the fully synthetic 4-aminoquinoline antimalarials — notably chloroquine and later amodiaquine<sup>10–12</sup>, which are inexpensive and administered over 3 days.

Amodiaquine use has been limited since the mid 1980s after it was linked causally to the occurrence of occasional agranulocytosis in adult travellers taking the drug prophylactically<sup>5</sup>. But because amodiaquine retains a high degree of efficacy against all but the most highly chloroquine-resistant strains, there has been a recent increase in its use<sup>5</sup>. Mefloquine and halofantrine are structurally related drugs that are active against chloroquine-resistant strains, but resistance can develop rapidly to each of these drugs<sup>2,13</sup>. Additional limitations include occasional neuropsychiatric disturbances for mefloquine and the fact that halofantrine is strongly contra-indicated in people with a history of heart disease<sup>5</sup>.

### Artemisinins

Several semisynthetic derivatives of artemisinin — the active ingredient of the Chinese herb 'qinghao' (*Artemisia annua*), which was used traditionally for treating fevers — have been used increasingly over the past two decades<sup>5,7,14–17</sup>. These derivatives (Box 1) include

artemether, arteether and artesunate, which are all metabolized to dihydroartemisinin — the main active agent in the body. These drugs are fast acting and act against gametocytes, the sexual stages of the parasite that infect mosquitoes.

The short half-lives of both the parent semisynthetic derivatives and the dihydroartemisinin metabolite necessitate treatment over 5–7 days when these compounds are used alone. They are therefore being used increasingly in combination with longer half-life drugs to reduce treatment time and increase individual compliance<sup>6</sup>. It is anticipated that rapid clearance of the parasites by artemisinin derivatives<sup>18</sup> will reduce the chances of resistance development to the partner drugs<sup>19</sup>. The combination of artesunate with mefloquine is recommended policy in some parts of Thailand<sup>5,20</sup>, and a study is nearing completion that involves numerous artesunate combinations in an attempt to identify the appropriate combinations for different epidemiological situations<sup>5,6</sup>.

The universal applicability and practicability of this approach still needs to be assessed fully, however, especially in Africa<sup>6,21</sup> where three main issues arise. First, what drug should be used for the longer half-life component of the combination? A partner drug with a very long half-life may be unsuitable for extended use in areas of intense transmission, where subsequent infections can occur before the drug has been cleared from the circulation but after the artemisinin derivative has been cleared; resistance is more likely to develop against the partner drug in such a situation. Second, compliance to complex drug regimens must be ensured against a backdrop of poor health-care infrastructure and patient supervision. The development of fixed-dose combinations (two or more compounds in a single tablet) might alleviate this problem. Third, the cost should be minimal. Although inexpensive by international standards, artemisinin derivatives are significantly more expensive than traditional antimalarials such as chloroquine and sulphadoxine/pyrimethamine.

The use of artemisinin derivatives has been negatively impacted by the observation that high parenteral doses of certain compounds can produce a limited, unique, selective brain stem neuronopathy in laboratory animals<sup>22</sup>. An analysis has concluded that these observations do not justify non-use of the compounds<sup>23</sup>, and no associated clinical symptoms have been detected<sup>24</sup>; however, limited preclinical data and quality assurance specifications for some products on the market continue to fuel discussion. Occasional allergic reactions to artesunate, the most widely used artemisinin derivative, have also been reported<sup>25</sup>.

The artemisinin fixed-dose combination product of lumefantrine/artemether, which is now registered widely, is among the most recently approved antimalarial drugs. The aryl alcohol lumefantrine is similar to mefloquine and halofantrine, and no neurotoxicity was seen during animal toxicology studies in preclinical development<sup>26</sup>. It is unclear whether a 2-day (4-dose) regimen or a 3-day (6-dose) regimen is the most appropriate treatment in Africa<sup>6</sup>. The need for higher dosing to ensure efficacy is supported by evidence of the cross-resistance of lumefantrine with mefloquine in both Thailand<sup>27</sup> and Cameroon<sup>28</sup>, and the dependence on food for optimal adsorption of lumefantrine from the gut<sup>29</sup>. With respect to the three issues on artemisinin combinations outlined above, it will be highly informative to discover how this drug performs in an African context.

#### Antifolates

The antifolate class of antimalarial drugs is not derived from plants and owes its origins to compounds generated through a knowledge of cell biology and synthetic medicinal chemistry. Fully reduced folate cofactors are essential for the key one-carbon transfer reactions needed for nucleotide biosynthesis and amino-acid metabolism<sup>30</sup>. At present, the most significant antifolate used to treat malaria is undoubtedly the combination of the 2,4-diaminopyrimidine pyrimethamine, an inhibitor of dihydrofolate reductase (DHFR), and sulphadoxine, a sulphonamide that interferes with the action of dihydroopterate synthase (DHPS), another enzyme in the folate pathway.

The two components of the sulphadoxine/pyrimethamine combination act as synergists with each other, enhancing their activity and reducing the propensity for resistance development. The long half-life of the components may also account for the fact that sulphadoxine/pyrimethamine is extremely useful for intermittent treatment in pregnancy<sup>31</sup>, although this link still remains to be proved. Unfortunately, resistance seems to develop rapidly when this combination is used extensively<sup>32,33</sup>. An additional concern that prevents the use of sulphadoxine/pyrimethamine as a prophylactic agent is that occasional hypersensitivity to the sulphur component may give rise to Stevens–Johnson syndrome — a toxic epidermal necrolysis that results in painful blistering of the skin<sup>5</sup>.

#### Atovaquone/proguanil

Atovaquone/proguanil is a fixed-dose combination whose development<sup>34</sup> shows similarity to that of sulphadoxine/pyrimethamine. The hydroxynaphthoquinone atovaquone interferes with mitochondrial electron transport. Rapid resistance develops against atovaquone<sup>34</sup> owing to a point mutation in cytochrome *c* reductase<sup>35</sup>. The addition of proguanil to atovaquone results in a synergistic activity that prevents the rapid development of resistance.

At present atovaquone/proguanil is used primarily as a prophylactic agent, and its price is too high for it to achieve widespread use in developing countries. One reason for this cost is the complexity of the synthetic route to atovaquone.

#### Antibiotics

Common antibiotics acting against bacterial protein synthesis such as tetracycline, doxycycline and clindamycin inhibit parasite growth and are being used increasingly in combination with other

antimalarial treatments to augment their activity<sup>5</sup>. In parts of south-east Asia, quinine plus tetracycline and quinine plus doxycycline are commonly used combinations. But their use in Africa is limited because both antibiotics are contra-indicated in children under 8 years of age<sup>5</sup>. Clindamycin is recommended in combination with other antimalarials in some situations.

These antibiotics are all thought to inhibit parasite growth through the inhibition of 'prokaryote-like' protein biosynthesis in the apicoplast — an organelle that is unique to apicomplexan parasites such as *Plasmodium* (refs 36,37; and see below).

#### Mechanisms associated with drug targets

Anti-infective drugs rely for their efficacy and specificity on their ability to interfere with aspects of metabolism that differ significantly from the human host. *Plasmodium* infects host erythrocytes during the phase of their life cycle that gives rise to the symptoms of malaria. Parasite survival in this environment requires several metabolic adaptations and innovations that render it susceptible to chemotherapeutic attack. This is manifest both in the mechanism of action of existing drugs and in the plethora of potential drug targets being identified, which is assisted by data from the malaria genome project (refs 38,39; see also <http://sites.huji.ac.il/malaria/>).

Many drug targets can be related to the functions of distinct organellar structures (ref. 40; and Box 2). Of particular interest are the lysosomal food vacuole (the site of extensive haemoglobin degradation), the apicoplast (a plastid organelle thought to originate from a green algal symbiont) and an acrystate mitochondrion with a limited electron transport system. Other aspects of metabolism are also important and are further highlighted.

#### Haemoglobin degradation in the food vacuole

During its 48-hour cycle of invasion, growth and release from an infected erythrocyte, the malaria parasite degrades up to 80% of the haemoglobin in the host cell<sup>41</sup>. This degradation occurs in a lysosomal food vacuole and involves aspartic proteases (plasmepsins)<sup>42</sup>, the cysteine protease falcipain 2 (ref. 43), and many peptidases including a metallopeptidase<sup>44</sup>. This results in the release of large amounts of Fe(II) haem, which is rapidly oxidized to Fe(III) haematin and sequestered as an inert pigment called haemozoin<sup>41</sup> that comprises a structured lattice of aggregated haem dimers<sup>45</sup>. Both the quinoline and aryl alcohol antimalarials<sup>46–50</sup> and the artemisinins and other antimalarial peroxides<sup>17,51</sup> are concentrated in the food vacuole and are thought to exert their activity through interaction with haem.

The quinolines are thought to disrupt or prevent effective formation of haemozoin by binding to haem through  $\pi$ – $\pi$  stacking of their planar aromatic structures<sup>52</sup>, resulting in haem-mediated toxicity to the parasite. This may occur by inducing lipid peroxidation<sup>53</sup>, although there are other explanations<sup>54</sup>. The artemisinins undergo oxidoreductive cleavage of their peroxide bond in the food vacuole, most probably through interaction with Fe(II) haem<sup>17,51</sup>. This generates fatal free-radical-induced damage to the parasite; however, the exact mechanisms by which free radicals are generated and the mechanism of parasite death are still matters of debate<sup>55</sup>.

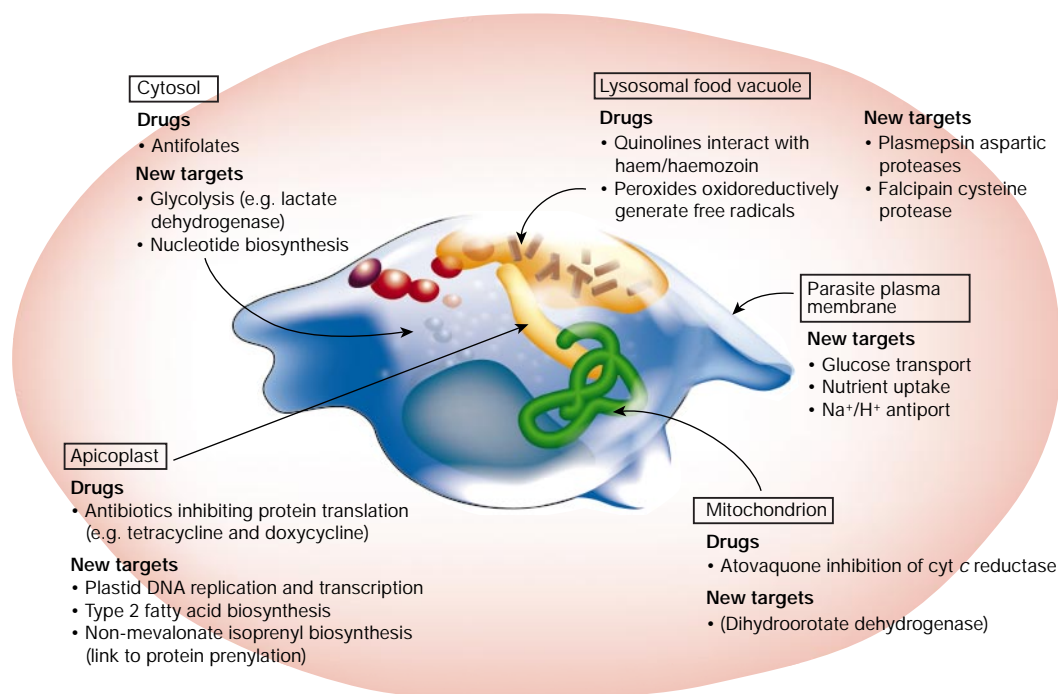
The lack of an enzyme drug target for both quinoline and artemisinin antimalarials is probably a chief reason why resistance development to these drugs is relatively slow<sup>2,33</sup>. For both classes of drug, resistance may be associated with mutations in transporters that affect drug access to the food vacuole. Data from genetic linkage experiments, transfection experiments and field monitoring of chloroquine-resistant parasites strongly suggest that *P. falciparum* resistance to chloroquine, and potentially to some other 4-aminoquinolines, is linked to the putative chloroquine resistance transporter gene *PfCRT*<sup>66</sup>. But this gene is not associated with chloroquine resistance in *P. vivax*<sup>57</sup>.

A second gene encoding a P-glycoprotein, *mdr1*, has been linked to the altered susceptibility of parasites to mefloquine, halofantrine and the artemisinins in cell-culture studies of a genetic cross<sup>58</sup> and

## Box 2

## Sites of drug action and new drug targets

Diagram of *P. falciparum* trophozoite residing in an erythrocyte. The main organelles that are associated with drug targets are highlighted, drawing attention both to sites of current antimalarial drug action and new targets that are under investigation. The concept for this representation is derived from the trophozoite illustrations of Bannister<sup>40</sup>.



through transfection of mutant genes from resistant strains into drug-sensitive parasites<sup>59</sup>. Amplification of this gene may also contribute significantly to resistance to mefloquine and halofantrine<sup>60</sup>. Despite these data, the association between *Pfmdr1* and resistance to mefloquine, halofantrine and quinine remains incomplete, suggesting that other genetic factors, including *PfCRT*, are involved<sup>13</sup>. It should also be noted that although mutations in *Pfmdr1* result in reduced sensitivity of parasites to artemisinin derivatives, no significant clinical drug resistance has been observed against the artemisinins.

Haemoglobin degradation and interaction with haem remain fruitful areas for drug discovery. Relatively small changes in quinoline structure can enhance compound uptake into the food vacuole of chloroquine-resistant parasites<sup>10,61</sup>. Efforts are currently directed towards short-chain chloroquine analogues, bisquinolines and analogues of amodiaquine that lack the ability to form a toxic metabolite. Significantly, pyronaridine — a 4-aminoquinoline developed originally in China — is undergoing development in combination with artesunate. Molecules that inhibit parasite growth through binding to haem in an analogous way to the binding of quinolines are also being sought through high-throughput screening<sup>62</sup>.

Several molecules have been identified that enhance the activity of chloroquine against chloroquine-resistant strains, most probably by enhancing the uptake of chloroquine into the parasite food vacuole<sup>13,63,64</sup>. A study in Nigeria in which chloroquine was combined with chlorpheniramine<sup>65</sup> has suggested that this approach may have clinical application, but further evaluation is needed.

Research chemists are attempting to identify semisynthetic and fully synthetic endoperoxides that will retain the activity of the artemisinin derivatives, but overcome some of their deficiencies, notably their short half-life and the residual fears of potential neurotoxicity<sup>16,66–69</sup>. Unexploited targets located in the food vacuole include the proteases associated with haemoglobin degradation, namely plasmepsin aspartic proteases<sup>41,42</sup> and the cysteine protease falcipain 2 (ref. 43). In both cases, malaria chemotherapy might

benefit from the intense medicinal chemistry efforts under way in the pharmaceutical industry, including the development of inhibitors against HIV aspartic protease and the aspartic protease renin (an enzyme associated with hypertension), and interest in the cysteine proteinase cathepsin K (which is implicated in osteoporosis). The challenge to chemists working in this area is to identify simple, non-peptidic structures with good oral bioavailability and low costs. Evidence that inhibitors of aspartic proteases and cysteine proteases are synergistic in their efficacy against *P. falciparum* in culture provides further opportunity in this area<sup>41</sup>.

#### Apicoplast metabolism and drug targets

The malaria genome project has made a major contribution to elucidating the metabolism of the apicoplast<sup>70</sup>. This plastid organelle contains a 35-kilobase circular genome that encodes elements of a prokaryotic transcription and translation system<sup>71</sup>, suggesting that this organelle is the target of the clinically used antimalarial antibiotics tetracycline, doxycycline and clindamycin, which target prokaryotic translation<sup>36,37</sup>. Preclinical work has also shown that malaria parasites are susceptible to prokaryotic transcriptional inhibitors such as rifampicin and DNA gyrase inhibitors such as the quinolones<sup>36,37</sup>.

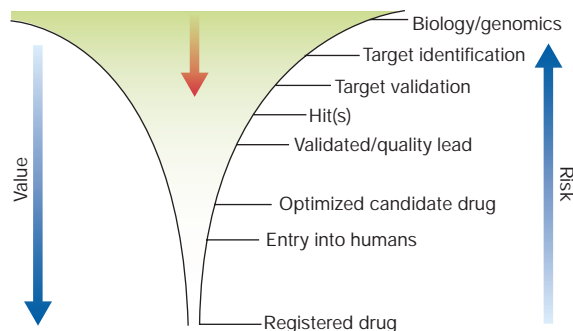
These antibiotics tend to kill the parasite slowly, but a combination of *Toxoplasma* molecular genetics and *P. falciparum* genomic information has facilitated the discovery of several key metabolic pathways in the apicoplast, the disruption of which results in a more rapid cell death. The first of these is type II fatty acid biosynthesis<sup>72</sup>, a pathway that is often associated with plant eukaryotes and bacteria. Several lead molecules have been identified in this pathway<sup>37</sup>: thiolactomycin inhibits the condensing enzymes Fab B, Fab F and Fab H in *Escherichia coli* and inhibits *P. falciparum* growth; triclosan is active against parasite growth in cell-culture and rodent models through inhibition of enoyl-acyl-carrier protein reductase<sup>73</sup>.

Another enzyme pathway identified through genomics and now firmly associated with the apicoplast is the non-mevalonate pathway that leads to synthesis of isopentenyl diphosphate subunits.

## Box 3

## Drug development funnel

The funnel provides an overview of the process of drug discovery and development. Many possibilities exist in terms of potential targets, but only a limited number of targets are valid and even fewer generate a chemical compound that justifies serious medicinal chemistry. Few compounds achieve drug candidate status and are worthy of testing in humans, and only a few of these are ultimately registered as a drug. As we move down the funnel, the cost of research on an individual project increases because of the more intensive work that is required to determine preclinical and clinical safety and efficacy, and to produce, manufacture and register the drug. Appropriate selection of which projects move forward and which are abandoned is the key to R&D success. As we move down the funnel, the risk of the project failing decreases and its value increases. The cost of an individual project proceeding through this process probably amounts to tens of millions of dollars. Taking into account the number of projects that fail, the cost of a success may be of the order of hundreds of millions of dollars.



Moreover, fosmidomycin, an inhibitor of 1-deoxy-D-xylulose-5-phosphate synthase that was identified in an industrial antibacterial drug discovery programme, inhibits effectively the malarial enzyme and parasite growth<sup>74</sup>, and offers hope either as an antimalarial agent in its own right or as a lead compound. The isopentenyl diphosphate generated by this pathway is also important for protein farnesylation. This is significant because the human protein farnesyltransferases have been investigated by pharmaceutical companies as anticancer drug targets. Recent studies have identified inhibitors of protein farnesyltransferase as potential leads for new antimalarials<sup>75</sup>.

Although the science underlying the enzymes involved in apicoplast metabolism is exciting, it is important to remember that when other enzyme inhibitors, namely pyrimethamine and atovaquone, were used alone in chemotherapy, resistance emerged rapidly. Hopefully, inhibitors of the different pathways of the apicoplast act as synergists in a manner similar to that of the antifolates and atovaquone/proguanil, and lead ultimately to combination treatments of high medical value.

#### Mitochondrion electron transport

The *Plasmodium* mitochondrion is unusual morphologically<sup>40</sup>. *Plasmodium* parasites reside in an oxygen-deficient environment and rely on glycolysis for ATP production. Thus, the mitochondrion has no oxidative phosphorylation activity and has an incomplete electron transport chain that provides certain redox reactions of metabolic significance. One such reaction is the coupling of cytochrome *c* reductase (cytochrome *b*/cytochrome *c1* complex) to dihydroorotate dehydrogenase, a key enzyme in nucleotide biosynthesis. Atovaquone is known to inhibit electron transport at this point<sup>76</sup>. Similar to experiences with the DHFR inhibitor pyrimethamine, resistance has been found to develop rapidly owing to single-point mutations in cytochrome *b* (ref. 35). This has been

addressed by combining atovaquone with proguanil, which acts in synergy as the prodrug with atovaquone<sup>34,35</sup>.

Other classes of compound are also known to inhibit cytochrome *c* reductase, notably the  $\beta$ -methoxyacrylates<sup>77</sup>. Compounds from this class are active against atovaquone-resistant strains, but resistance develops very rapidly against them in rodent models of malaria (H. Matile, personal communication).

#### Folate pathway

The genetic basis of resistance to the antifolate antimalarials is probably the best understood of all the drug-resistance mechanisms described to date<sup>3,4,78</sup>. High-level pyrimethamine resistance results from the accumulation of mutations in DHFR, principally at codons 108, 59, 51 and 164, where allelic variation gives rise to the mutations Ser108→Asn, Cys59→Arg, Asn51→Ile and Ile164→Leu. Mutations occurring in DHPS at codons 436, 437, 581 and 613 in cultured parasite lines broadly correlate with estimated levels of sulphadoxine resistance. Variability in codon 540 is also observed commonly in field samples. Notably, although uptake of exogenous folate bypasses DHPS, pyrimethamine restores sulphadoxine sensitivity at concentrations much lower than those required to inhibit DHFR, suggesting that pyrimethamine may have additional antiparasitic effects.

Several medicinal chemistry efforts are being directed at identifying improved inhibitors of DHFR that overcome resistance to pyrimethamine. Biguanides related to proguanil, which are metabolized to active triazines, offer the most immediately promising results. Chlorproguanil, which is metabolized to chlorcycloguanil, retains activity against all but the most highly pyrimethamine-resistant strains, and it is anticipated that a fixed-dose combination of this compound with dapsone should achieve registration early in 2002 (ref. 79). Efforts are under way to combine chlorproguanil and dapsone with artesunate to lessen the chances of drug resistance development. Another set of potential biguanide prodrugs also shows promise<sup>80</sup>. Our understanding of how DHFR mutations confer resistance, our ability to model the DHFR active site, and the ease of the DHFR assay suggest that further improved inhibitors are achievable. Many other enzymes in the folate pathway may also be potential drug targets<sup>38,81</sup>.

#### Other promising areas for drug discovery

Although it is not possible to discuss all of the many scientific opportunities that are presenting themselves for malaria drug discovery, several other areas deserve to be highlighted. Because malaria parasites are microaerophilic homolactate fermenters and rely on glycolysis for ATP production, lactate dehydrogenase is being explored as a drug target<sup>82</sup>. Because the parasite requires enormous amounts of glucose uptake from the host to support its growth, the identification of a glucose transporter by functional transfection of the gene into *Xenopus* oocytes is potentially significant<sup>83</sup>.

Other transport studies are further identifying potential drug targets<sup>84</sup>, including an anion-selective channel that is responsible for transporting solutes and nutrients<sup>85</sup>. Proteases involved in erythrocyte invasion also offer potential<sup>86</sup>, as do some highly active compounds that may interfere with phospholipid biosynthesis<sup>87</sup>. Cell signalling pathways, particularly through protein kinases, offer huge potential for drug discovery<sup>88</sup>, and there is a wealth of medicinal chemistry experience available in this area from industry. Until protein kinases can be linked to specific functions, however, it will be difficult to assign a relevance to particular genes for drug discovery.

#### Power of genomics

The power of genomics and molecular genetics has underpinned many of the biological advances mentioned here, from determining the mechanisms of chloroquine drug resistance to establishing the metabolic pathways associated with the apicoplast. The power that the full malaria genome sequence will bring and the genomic tools at our disposal have been dealt with in depth by others (ref. 89; and see

Hoffman *et al.*, pp. 702–709, in this issue). There is already industrial evidence supporting the power of genomic approaches in microbial drug discovery<sup>90</sup>. Bioinformatics tools can help predict the structure and function of gene products<sup>91</sup>. Combined with the power of ‘virtually’ docking compounds into modelled protein structures and of robotics for high-throughput screening, the speed with which many compounds can be tested against a protein target is ceasing to be a limiting factor.

For malaria, the main bottleneck in the functional genomics of *P. falciparum* is the limitation of our transfection systems, although many examples of successful transfection experiments now exist<sup>89,92</sup> and improvements are being made continuously. Currently, it takes about 3–4 weeks to obtain a single transfectant owing to low transfection rates and the (relatively) slow growth of the parasite. Only single crossover insertions have been observed so far in *P. falciparum*<sup>92</sup>. Transfection capabilities are also available for some model animal systems, including *Plasmodium berghei*<sup>89,93</sup>. In many cases, the enhanced efficiencies of these processes allow double crossovers and gene replacement to occur.

We also gain insight into *P. falciparum* biology by using molecular genetics tools developed for *Toxoplasma gondii*, a related apicomplexan parasite<sup>72</sup> that has the additional advantage of allowing non-homologous integration. The ability to perform knockout experiments more routinely and gene-replacement experiments on a larger scale in *P. falciparum* would advance the field greatly. Of particular importance for drug discovery is the development of inducible knockout techniques, such as tetracycline-inducible systems and RNA inhibition, that can transiently and selectively knockout gene function to allow observation of the resultant phenotype.

Transfection technology will produce numerous tools to assist drug discovery and development. The replacement of a gene encoding a drug target in *P. berghei* with its *P. falciparum* homologue is currently being developed (D. Fidock and W. Jacobs, personal

communication) and deserves comment. A chief difficulty in drug discovery is moving from molecular assays, to cellular assays, to appropriate animal models. Unfortunately for malaria drug discovery, the human malaria parasites do not infect rodents, and specific rodent malaria parasites such as *P. berghei* have to be used. For targets associated with haem, such as the quinolines and the peroxides, the rodent malaria parasite *P. berghei* is an excellent model. But for enzyme targets in which there may be differences in protein sequence, structure and inhibitor specificity, this ceases to be the case. In the absence of sufficient similarity between the enzymes of *P. berghei* and *P. falciparum*, one has either to rely on cell-culture data combined with rapid pharmacokinetic evaluation or to move to much more expensive (and low-throughput) monkey models that can sustain infection by *P. falciparum*. The ability to test inhibitors against a *P. falciparum* (or *P. vivax*) enzyme in a rodent system would greatly facilitate rapid compound evaluation and feedback to medicinal chemists on aspects such as oral bioavailability and efficacy.

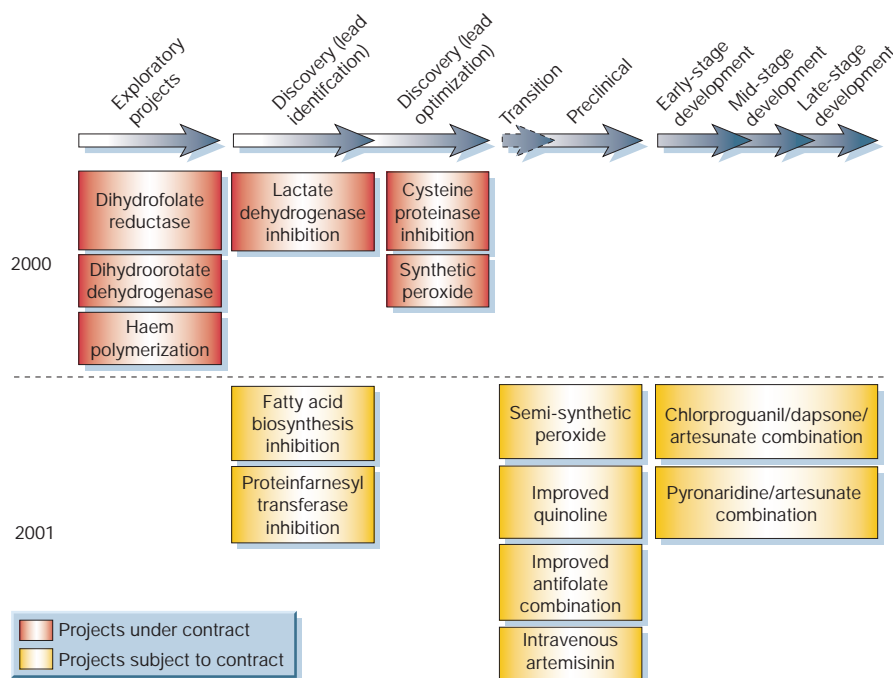
### Focused drug discovery and development

When discussing issues relating to drug discovery and development, it is necessary to keep in mind the many disciplines and resources that have to come together to deliver success<sup>94</sup>. This should be coupled with the understanding that success is by no means guaranteed. Box 3 shows a ‘funnel’ model of the discovery and development process. Genomics can provide many potential molecular targets, but only those targets that can be readily manipulated and tested are likely to provide an opportunity to discover a lead molecule, for example by high-throughput screening, that is worth taking on to a full medicinal chemistry project. Thus, many potential targets will never be advanced because no chemistry lead is identified. Once a chemistry effort is started, many factors need to be considered over and above improving enzyme inhibition and efficacy against the parasite in culture. The molecules finally selected for development must also be

#### Box 4

#### Significance of developing a portfolio of projects

An example of a drug research and development portfolio taken from the 2001 Medicines for Malaria Venture annual report (<http://www.mmv.org>). The arrows refer to the discovery and development process that takes about 10–15 years. It comprises an exploratory phase in which a project attempts to develop the tools and lead molecules necessary for a concerted discovery effort; a discovery phase in which lead compounds are better characterized, developed and optimized using medicinal chemistry; a transition and preclinical development phase in which candidate compounds undergo extensive analysis with respect to toxicity, adsorption, pharmacokinetics and formulation; and finally the various clinical phases during which continued manufacturing, production and toxicology studies are undertaken. Some of the projects for 2001 are still subject to contract. The Medicines for Malaria Venture has calculated that it needs to double the current portfolio to ensure the registration of one new drug every 5 years.



easy to manufacture (low cost is crucial for antimalarials), stable, readily formulated, bioavailable (that is, extensively adsorbed from the gut and avoiding first pass metabolism in the liver to achieve effective concentrations in the systemic circulation), have an appropriate half-life, and not show any overt toxicity.

It is essential that academic scientists better understand these issues as they are increasingly having to take the lead in drug discovery efforts against malaria and other neglected diseases. An analysis of the reasons why candidate drugs fail to achieve registration and reach the market has been undertaken recently<sup>95</sup>. In 39% of cases, drugs failed after entering development because of 'biopharmaceutical' issues such as oral bioavailability and formulation, and in 21% of cases because of toxicity. These issues are equally as important as drug efficacy, for which 29% of cases failed.

Over the past 5 years, progress has been made in defining rules to assist chemists in the synthesis of compounds that will possess adequate properties for formulation and bioavailability. This is perhaps best embodied in variations of Lipinski's rules<sup>96</sup>, which define appropriate parameters for optimizing solubility and membrane permeability such as  $\log P$  (where  $P$  is the partition coefficient between octanol and water), molecular weight, and the number of hydrogen bond donor and/or acceptor groups. Appropriate modelling and analysis of compounds for these parameters is ultimately as important to obtaining a drug as the molecular modelling of compounds into the active sites of enzymes. Information technology and sophisticated databases also enable chemists to identify molecular groups that are likely to be metabolically labile or that have associated toxicity.

Successful drug discovery and development requires manpower, financial resources and management. The skills required and the experience of bringing these areas of expertise together in a team exist primarily in the pharmaceutical industry. In recognition of this key industrial role, the Medicines for Malaria Venture was established (<http://www.mmv.org>). This organization builds on the experiences of the Special Program for Research and Training in Tropical Diseases (<http://www.who.int/tdr>) and operates in concert with the Roll Back Malaria campaign of the WHO (<http://www.rbm.who.int>). It seeks through appropriate funding to bring together teams of scientists from both academic and industrial backgrounds to form powerful drug discovery and development project teams. The projects that are funded combine to generate a portfolio (Box 4) that over time is expected to deliver new antimalarials at regular and optimum intervals.

Current drug discovery and development efforts centre on identifying molecules that will be active in treating uncomplicated malaria. The ideal product profile comprises orally active compounds that can cure the disease with a 3-day regimen using once-a-day dosing. As a strong portfolio develops, however, it is anticipated that other, more specific malarial indications may be targeted, such as adjunct treatments to improve the outcome of severe malaria cases; intermittent treatment of malaria during pregnancy to protect both the mother from the disease and the unborn child from a higher risk of being born underweight<sup>31</sup>; and long half-life drugs to treat malaria with a single dose in complex emergency situations. There is also the prospect of combining compounds from several projects into combination products. This could both enhance efficacy and reduce the likelihood of drug resistance development.

### Drug access

Good drugs are only useful if they are made available to the people who need them and are used properly. Thus, the availability of inexpensive compounds, sound policies on their use, strong healthcare infrastructure, and sufficient funds to purchase the drugs that are needed are all vital<sup>5,79</sup>. The political will and momentum that are being generated in the countries worst affected by the disease through the WHO-led global campaign to Roll Back Malaria<sup>97</sup>, combined with renewed efforts to obtain both sufficient global support and

funds to purchase drugs<sup>98</sup> and the increased science funding going into malaria drug discovery and development, should result in a significant reduction in the malaria disease burden over the coming years and decades.

Progress in drug discovery and development should deliver a larger choice of drugs to treat malaria. This will reduce a current dilemma that faces many malaria control agencies. Because of the lack of alternatives they find it difficult to balance the need to distribute the most effective drugs widely against the need to minimize resistance development through controlled use. The world needs to reach a situation in which many effective, appropriate and affordable antimalarial drugs are in reserve to meet the challenge of resistance as and when it arises. □

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#### Acknowledgements

I thank W. Jacobs and D. Fidock for allowing me to describe their concept for transfecting genes from human *Plasmodium* species into rodent *Plasmodium* species; T. Sukwa for discussions; and D. Fidock for reading the manuscript.