CHAPTER 1

Malaria: New Medicines for its Control and Eradication

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1.1 Introduction

Malaria is caused by protozoan parasites of the genus *Plasmodium* that infect and destroy red blood cells, leading to fever, severe anaemia and, if untreated, cerebral malaria and death. *Plasmodium falciparum* is the dominant species in sub-Saharan Africa, and is responsible for almost one million deaths each year.\(^1\) The disease burden is heaviest in sub-Saharan African children under 3 years old (who have frequent attacks and little immunological protection), and also in expectant mothers.\(^1\) Malaria is both a cause and a consequence of poverty: in countries with intense malaria transmission, the economic impact of the disease results in a slowing of economic growth of 1.3% per year\(^1\), translating to a reduction of the Gross Domestic Product in sub-Saharan Africa estimated to be US$12 billion per year.\(^2\)

The global fight to control malaria requires a multifaceted approach. At present, we have a wide range of effective tools. Medicines can be used to prevent as well as to cure, especially in vulnerable populations such as infants\(^3\) or pregnant women.\(^4\) Insecticides and larvicidal spraying and the use of insecticide-impregnated bed nets to protect against infection by mosquitoes have dramatically increased in recent years. This success brings with it the need for
the development of the next generation of insecticides, since resistance to the current gold standard, the pyrethroids, is already an issue. Developing a vaccine is proving especially challenging, as the parasite has sophisticated mechanisms for avoiding the host immune system. The best candidate currently is GSK 257049, known as RTS.S/AS202, where phase III trials are expected to finish in July 2013. Phase II studies suggest that it will reduce risk of clinical malaria, and decrease mortality in severe malaria by 50%.

In November 2007, the Bill and Melinda Gates Foundation set an agenda with the final goal of completely eradicating malaria, an objective supported by both the World Health Organization (WHO) and its Roll Back Malaria (RBM) partnership. Commentators have described this objective as ‘worthy, challenging, and just possible’, but one which must be pursued with balance, humility, and rigorous analysis.

The addition of this new goal has implications for the global malaria R&D agenda, which have been discussed in a variety of different working groups. Future antimalarial medicines must not only be able to treat the asexual blood stages of *P. falciparum*, but also to block the transmission of the parasite to other persons via the mosquito vector and, in the case of *P. vivax* infection, to target the dormant liver-stage of the parasite. In this chapter, we discuss the current pipeline of antimalarial medicines, and the target product profiles for the generation of such products.

### 1.2 The Challenges of the Different *Plasmodium* Species

Four main species of the malaria parasite infect humans. *P. falciparum* is responsible for the vast majority of the malaria-linked deaths in sub-Saharan Africa and is therefore the most important target. *P. vivax* constitutes as much as 25–40% of the global malaria burden, particularly in South and Southeast Asia, and Central and South America. It does not normally progress to cerebral malaria, and has been traditionally labelled benign. However, *P. vivax* causes a greater host inflammatory response than *P. falciparum* at equivalent parasitaemia. Mortality from *P. vivax* is most likely underreported, as recent analyses in Papua (Indonesia), have shown similar mortality figures in children to those found with *P. falciparum*.

Medicines that are active on the asexual erythrocyte stages of *P. falciparum*, such as the artemisinin-based combination therapies (ACTs), are assumed to be fully active against the other species. The formal clinical database supporting this assumption is relatively thin but is well supported by empirical observation. Historically, mixed infections of *P. falciparum* and *P. vivax* are rarely reported, possibly because *P. falciparum* suppresses the development of *P. vivax*, but PCR detection methods have shown that these can be as high as 30%. The other two species are *P. malariae* and *P. ovale*. Currently, these are diagnosed by microscopy, and represent a small percentage of infections. Diagnosis based on Polymerase Chain Reaction (PCR) will undoubtedly lead
to a re-evaluation of the presence of mixed infections, since it is able to quantify low parasite numbers, and is often more definitive as a diagnostic.

From a treatment and eradication perspective, there are 3 key differences between the species. The first difference occurs in the liver. Following infection of the patient, parasites rapidly progress to infect hepatocytes, undergo asexual schizogony, and release large numbers of merozoites into the host bloodstream. In *P. vivax* and *P. ovale*, some of the liver parasites become dormant (a form known as the hypnozoite).\(^{15,16}\) These forms can be reactivated after periods that vary from 3 weeks to several years, dependent on the strain of parasite and the status of the host. Unless the hypnozoites are eliminated, malaria will continue to relapse periodically. Since *P. vivax* transmission is rarely intense, activation of hypnozoites is thought to be a major contributor to disease frequency.

The second difference between the species is in the time taken for the parasite to replicate in the host. The time between febrile paroxysms varies from around 48 hours for *P. falciparum* and *P. vivax* to 72 hours for the more benign *P. malariae*. There has been a recent interest in a fifth species, *P. knowlesi*, a parasite of Old World monkeys, now known to infect humans.\(^{17}\) PCR methods show that it is often misdiagnosed as *P. malariae* infection, which is usually uncomplicated and has low parasitaemia. However, *P. knowlesi* replicates every 24 h, and so is potentially life-threatening if not treated expeditiously.\(^{18}\) Therapeutically, the challenge in this case is to have a therapy with a rapid onset of action.

The third is the timing of the appearance of gametocytes in the blood stream.\(^{19}\) In *P. falciparum*, the gametocytes do not appear until several days after the initial parasitaemia and fever, whereas in *P. vivax* they appear concurrently or even before asexual parasites. An ideal treatment for blood stages of *P. vivax* must be able to kill existing gametocytes, rather than simply preventing them from differentiating (see Figure 1.1).

### 1.3 Currently Available Antimalarials

The roots of most antimalarial treatments are based on three natural products: quinine, lapinone and artemisinin.\(^{20}\) In each case the natural product was known to have some activity from traditional medicine, and was isolated, shown to have some activity and then this activity was improved by classical medicinal chemistry.

The first widely used antimalarial drug was quinine, a natural product extracted from the bark of the tree *Cinchona calisaya*. It causes parasite death by blocking the polymerisation of the toxic by-product of haemoglobin degradation, haem, into insoluble and non-toxic pigment granules, resulting in cell lysis and parasite cell autodigestion.\(^{21}\) This means that the parasite is not able to generate resistance at the target site: the molecular target is a non-mutatable chemical reaction. Quinine itself is active when given 3 times a day for 7 days. Initial attempts to synthesise quinine led to the synthesis of dye
substances, some of which are actually antimalarials in their own right, such as methylene blue. Later work produced chloroquine, a 4-aminoquinoline, which was the mainstay of malaria prophylaxis and treatment for the second half of the 20th century. This also has the advantage that its electronics allow it to

Figure 1.1  Life cycle of the malaria parasite.
1 Sporozoites are injected into humans with the saliva of a female *Anopheles* mosquito. 2 They are rapidly taken up into the liver, passing through Kupffer cells to hepatocytes. 3 Here the parasites develop to form several thousand merozoites. 4 In *P. vivax* and *P. ovale* only, some liver-stage parasites remain as a dormant form, or hypnozoite, characterised histopathologically as a small uninucleate parasite, which remains dormant for a few weeks, or up to several years. These species can therefore start a new cycle of asexual infection even without a mosquito bite. 5 The liver cells rupture and the merozoites are released into the blood, rapidly invading erythrocytes. 6 The intra-erythrocytic parasites replicate synchronously, leading to the classical cycle of fever observed clinically. 7 Some merozoite-infected red cells develop into male and female gametocytes. In *P. falciparum*, these are formed in the later stages of infection, whereas, in *P. vivax*, they are formed at the same time as the asexual stages. 8 Gametocytes are taken up into the female mosquito gut during the blood meal. 9 The male gametocytes are activated (exflagellation) and fuse with the female gametocytes to form diploid ookinetes. These ookinetes migrate to the mid-gut of the insect, pass through its walls and form the oocysts. 10 Meiotic division occurs and sporozoites are formed. 11 These then migrate to the salivary glands of the mosquito. Taken from Wells et al.91
selectively concentrate into the food vacuole. Further synthetic work has yielded many more aminoquinolines and related amino-alcohols such as amodiaquine, mefloquine, halofantrine, lumefantrine, piperaquine and pyrnonaridine. These medicines are characterised by a large volume of distribution and a long half life (terminal half lives of over 5 weeks are reported for chloroquine). They also require reasonably high doses (total dose of between 1250 and 2500 mg for adults, normally split into three daily doses. They have been linked to cardiac safety issues: at high doses prolongation of the QTc interval has been seen with some medicines, and indeed this led to the withdrawal of halofantrine by SmithKlineBeecham. Two more recent medicines in this class are Ferroquine (SSR97193, by sanofi-aventis, in phase II) and naphthoquine (launched as ARCO, by Kunming Pharmaceutical Corporation). Both have long half lives, and the clinical question is whether their therapeutic window is large enough to support a single dose as a cure. Naphthoquine is administered as a 400 mg single dose with artemisinin and is reported to be safe22 and effective in small scale clinical trials, 23 although the key data on QTc prolongation are currently not available. Ferroquine is currently in phase II trials with a lowest adult dose of 100 mg. An alternative approach has been to produce chloroquines linked to molecules known to reverse the CQ resistance transporter.24 Although this is synthetically interesting, the challenge is still to show superiority in cardiovascular safety, which is far from trivial. In addition, molecules such as azithromycin have been shown to reverse chloroquine resistance clinically, 25 and so the challenge for a new molecule will always be to demonstrate additional benefit over known medicines.

Lapachol is a hydroxynaphthoquinone used to treat malaria and fevers in South America.26 It was initially reported in the 19th century. As part of the American war effort it was tested in P. lophurae infected ducks in 1943, and showed weak activity. A close synthetic derivative, lapinone, was also active. This was subsequently confirmed in patients with P. vivax27 by intravenous administration for 4 days. Solving the bioavailability issues led to the development of the orally bioavailable, metabolically stable molecule atovuqone,28 one of the active ingredients for Malarone, the current mainstay of antimalarial prophylaxis for travellers. Further work focussed on the 4-pyridones and led to the development of GSK932121.29 Work on this molecule was stopped in phase I after safety concerns with a pro-drug formulation. Hydroxynaphthoquinone and 4-pyridones target the cytochrome bc1 complex, and so in addition to the solubility/bioavailability and drug metabolism issues, there is clearly with such molecules a need to show selectivity against inhibition of the host electron transport. Recently, new inhibitors with selective bc1 inhibition have been reported based on an acridinone template WR 249685.30

The third approach is built on the discovery of the sesquiterpene lactone artemisinin (known as Qing hao su) in 1972 by Chinese scientists.31 It is an endoperoxide-containing natural product isolated from the leaves of the sweet wormwood, Artemisia annua. Derivatives of artemisinin were subsequently
shown to be more potent than the parent molecule, including dihydroartemisinin (DHA, believed to be the main active metabolite of all the derivatives), artemether, artemotil and artesunate (see Figure 1.2). Artemisinin itself is highly insoluble: chemical modification to artesunate increases oral bioavailability, and also makes it suitable for intravenous administration in severe malaria. The artemisinin derivatives are fully active against all existing drug-resistant strains of *P. falciparum*. Unlike all other antimalarial drugs, they act on all stages of the parasite intraerythrocytic life cycle and therefore rapidly kill all the blood stages of the parasite, resulting in the shortest fever and parasite clearance times of all such medicines. Furthermore, the artemisinins also kill gametocyte stages – thereby reducing transmission from humans to mosquitoes.

(i) The 4-aminoquinolines and amino-alcohols used in the treatment of uncomplicated malaria.

![Figure 1.2 Structures of key antimalarial compounds.](image-url)
(ii) The development of electron transport inhibitors. The natural product Lapinone was used as the design for Lapachol, which has limited oral bioavailability. Further work on this has yielded Atovaquone, which is part of the successful prophylactic, Malarone. Work to further improve the series has led to compounds such as WR 249685 from the Walter Reed Institute of Army Research, but so far no compounds have entered clinical development.

![Lapachol and Lapinone](image1)

![Atovaquone and WR249685](image2)

(iii) The artemisinins: artemisinin, dihydroartemisinin (DHA) the principle metabolite, the methyl ether, artemether, and the ethyl ether, arteether; and artesunate.

![Artemisinin, Dihydroartemisinin (DHA), Artemether, Arteether (Artemotil) and Artesunate](image3)

**Figure 1.2** Continued.

Not all antimalarial drugs can be traced back to natural products. Some were rationally designed following an antimetabolite approach. For example, malaria parasites are unable to salvage folate, but need this cofactor to synthesise tetrahydrofolate for methylation reactions. Inhibitors of dihydropteroate synthase (sulphonamides such as sulphadoxine) and dihydrofolate reductase (2,4-diaminopyrimidines such as pyrimethamine) are potent antimalarial drugs, especially when administered in combination.

For most of the second half of the 20th century, control of acute uncomplicated malaria caused by all four species of *Plasmodium* relied on chloroquine for first-line treatment and a combination of sulphadoxine and pyrimethamine (SP) as second-line treatment.
(iv) The pathway beyond artemisinin. The semi-synthetic derivative artemisone; the endoperoxide/4-aminoquinoline fusion compound Trioxaquine (SAR116242, also known as PA1103); the first generation endoperoxide OZ277 (now under development as Rbx11160); the next generation endoperoxide OZ439, an endoperoxide CDRI 97/98 (under development by IPCA); and a synthetic endoperoxide RKA182, with a distinctive tetraoxane.

![Chemical Structures](image)

(v) The current non-artemisinin containing combinations (NACTs): atovaquone-proguanil (Malarone), the proguanil is metabolised into cycloguanil, which acts synergistically and sulphadoxine-pyrimethamine (SP, Fansidar).

![Chemical Structures](image)

Figure 1.2  Continued.
1.4 Resistance

Resistance is a fact of life with antimalarial drugs, but the danger can be reduced by combination therapy. The frequency of mutations that might lead to resistance to drugs in *P. falciparum* is estimated at 1 in $10^{10}$ parasites.

(vi) The 8-aminoquinolines targeting *P. vivax* hypnozoites: pamaquine, primaquine and tafenoquine.

(vii) Spiroindolone NITD 609, the first clinical fruits of the whole cell screening strategy.

(viii) Natural products: identified from extracts which have been demonstrated to have activity in patients with clinically defined malaria (according to WHO guidelines).

![Chemical structures of pamaquine, primaquine, tafenoquine, NITD609, protopine, allocryptopine, berberine, and strictosamide.](image)

**Figure 1.2** Continued.

1.4 Resistance

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compared with a parasite burden of $10^{12} - 10^{13}$ in humans with severe malaria. Combining two medicines with different mechanisms of action lowers the probability that a resistant parasite will emerge and become established.\textsuperscript{33} Against this background, it is not surprising that in all cases, \textit{Plasmodium} strains resistant to current antimalarial drugs emerged. Resistance against drugs such as sulphadoxine-pyramethamine, where there are clear biological targets, arose more rapidly than with drugs such as chloroquine where the target is an immutuable chemical reaction and therefore other mechanisms, such as blockade of drug uptake, must be selected. Also, for reasons that are still not clear, resistance arose more rapidly in \textit{P. falciparum} than in \textit{P. vivax}. This might simply be an expression of less drug pressure, but this is by no means certain.

With the demise of chloroquine and SP because of drug resistance, in 2006 the WHO produced new treatment guidelines for uncomplicated \textit{P. falciparum} malaria: they recommended that the treatment of choice should be a combination of two or more antimalarials with different mechanisms of action.\textsuperscript{34} More than this, they suggested that artemisinin monotherapy should be withdrawn, to protect the class against the emergence of resistance – the first time such a suggestion had been made. The standard treatment rapidly became artemisinin-based combination therapies (ACTs), moving towards fixed dose (with both drugs in the same tablet, to prevent the artesenate being used as monotherapy). Artemether-lumefantrine, the first such fixed-dose artemisinin combination therapy developed to international standards of good practice, was launched by Novartis in 2001. Amodiaquine-artesunate was developed as a fixed-dose combination by the Drugs for Neglected Diseases Initiative (DNDi), and launched in 2008. The same year, a paediatric-friendly version of artemether-lumefantrine was launched as the result of a collaboration between Novartis and Medicines for Malaria Venture. In 2010, 82 million people were treated with Coartem or Coartem-D artemether-lumefantrine, and an additional 21 million treatments of generic artemether-lumefantrine were sold, mainly subsidised by the Affordable Medicines for Malaria Facility AMFm. The second most important combination by volume was amodiaquine-artesunate produced mainly by sanofi-aventis, with around 45 million courses of treatment supplied in 2010. So far, three generic producers of artemether-lumefantrine have been set up in Africa, and are able to supply drugs at the same price as Novartis ($0.30 for the smallest children, through to $1.20 for adults). Two other fixed-dose combinations are due to be launched over the next year: DHA-piperaquine (a collaboration between Sigma-Tau and Medicines for Malaria Venture) and pyronaridine-artesunate (a collaboration between Shin Poong and Medicines for Malaria Venture). Another version of DHA-piperaquine is available from Holley-Cotec. A fixed-dose combination of mefloquine-artesunate is available in Brazil from Drugs for Neglected Diseases initiative/Farmanguinhos/Fiocruz and in Europe from Mepha. A final combination of artemisinin (not the soluble artesunate) with napthoquine has been marketed by Kunming Pharmaceutical Corporation: although this has the advantage
on paper of being a treatment given in a single dose, or two doses in the same
day, there are few data around the long-term safety, and no clinical studies
have been carried out to determine good clinical practice. In summary, in
2010, over 150 million treatments for malaria were produced, enough for
60% of the cases of malaria identified globally. This is a tremendous step
forwards compared with 5 years ago, when less than 10% of malaria patients
were getting the best-quality treatments. (See Table 1.1).

One question we should ask is whether there are too many fixed-dose arte-


misinin combination medicines available or in late clinical development. The
clear answer is ‘no’: it is already apparent that there are clear differences
between these medicines: safety profiles are not the same, costs of goods sold
can vary considerably, shelf lives are different, not all have paediatric versions
and some have longer half lives, and so would give an advantage in terms of
post-treatment prophylaxis (how long after you are treated before you fall ill
again). This is important in some parts of Africa where children may have 10
episodes of malaria a year; it becomes less important in parts of Asia where the
transmission rate is much lower. Most importantly, cross-resistance patterns to
the non-artemisinin partner drug are not the same. If artemisinin resistance
becomes an issue, ACTs will still be clinically effective, but the resistance
pressure on the partner drugs will also increase. A wider range of partner drugs
is useful; but for now any new partners must bring significant advantages in
terms of cost, dose or safety, properties that are difficult to predict ahead of
pivotal studies.

1.5 Drugs for *Plasmodium vivax*

*P. vivax* malaria is generally still treatable with chloroquine, and so remains
WHO’s recommendation for first-line use against this species of *Plasmodium.*
However, some areas in South and Southeast Asia now harbour chloroquine-
resistant parasites. In these areas, WHO recommends the use of an ACT.
Pyronaridine-artesunate is the only fixed-dose ACT to date where *P vivax*
erthrocytic stages have been included in the label request, and pyronaridine
appears to be the most potent anti-vivax agent.

For *P. vivax* malaria, the particular challenge remains prevention of
relapse. Patients who have had all blood stages of the parasite killed can still
be re-infected by activation of the hypnozoite. Amongst the first generation
of synthetic drugs for malaria was the 8–aminoquinoline, pamaquine (Figure
1.2), introduced in 1926. It has anti-relapse activity but causes haemolysis
in patients who have a deficiency in the enzyme glucose 6-phosphate dehy-
drogenase (G6PD), meaning that ideally it should not be prescribed without
patient screening. This is a big problem in disease-endemic areas where up to
20% of the patients might have this deficiency. A second-generation
molecule, primaquine, was used initially on returnees from the Korean war,
where long-term relapses were extremely common. It is now the anti-relapse
drug of choice for *P. vivax* malaria. However, primaquine also causes
Table 1.1 Fixed-dose artemisinin combination therapies for treatment of malaria already available or in late-stage development.

<table>
<thead>
<tr>
<th>Partnership</th>
<th>Novartis, MMV</th>
<th>Sanofi-aventis, DNDi, b MMVc</th>
<th>Sigma Tau, MMV, Pfizerc</th>
<th>Shin Poong, MMV, Pfizerd</th>
<th>Farmanguinos, DNDi, Mepha, Cipla, d</th>
<th>Kunming Pharmaceutical Corporation KPC ARCO</th>
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</thead>
<tbody>
<tr>
<td>Trade Name</td>
<td>Coartem®/Coartem-D®</td>
<td>Coarsucam® 4Q’08</td>
<td>Eurartesim® 3Q’11</td>
<td>Pyramax® 1Q’12</td>
<td>— 2Q’08</td>
<td>— 2Q’08</td>
</tr>
<tr>
<td>Launch Date</td>
<td>1Q’01/1Q’09e</td>
<td>4Q’08</td>
<td>3Q’11</td>
<td>1Q’12</td>
<td>2Q’08</td>
<td>?</td>
</tr>
<tr>
<td>Key Strengths</td>
<td>Market leader with over 350 million treatments given. Excellent safety data. Paediatric formulation. WHO Prequalified</td>
<td>Once a day dosing. First-line therapy in francophone Africa. WHO prequalified</td>
<td>Once per day therapy. Long terminal half life of piperaquine. 80% patients are protected against re-infection at 42 days</td>
<td>Once per day therapy. Clinical data &amp; registration also for P. vivax malaria. Potential to combine with primaquine for radical cure. Paediatric formulation available</td>
<td>Once a day therapy. Satisfactory safety record in Thailand of non-fixed combination. Successful treatment of P. vivax malaria in chloroquine-resistant areas</td>
<td>Single dose of 1 g artemisinin, and 400 mg Naphthoquine; maybe split over one day</td>
</tr>
<tr>
<td>Key Weaknesses</td>
<td>Twice per day treatment. Low bioavailability, potential dependence on fatty foods</td>
<td>Resistance (to amodiaquine) can compromise efficacy. Reputaion for significant nausea. No approval by stringent Regulatory Authority</td>
<td>Stability: DHA least stable of the artemisinins; Paediatric formulation still in development</td>
<td>Mixture of two diastereoisomers. Psychiatric and GI adverse events. No approval by stringent Regulatory Authority. Difficult to use in countries where Mefloquine is used as prophylaxis, currently expensive ($2.50).</td>
<td>No GCP clinical studies, little long-term safety data. No approval by Stringent regulatory authority</td>
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</tr>
<tr>
<td>Market size (Number of treatments sold in 2010)</td>
<td>82 million total, (37 million Coartem®). Estimated 21 million additional generic Artemether Lumefantrine</td>
<td>45 million (fixed dose) combination</td>
<td>2 million</td>
<td>None (launch date in 2011)</td>
<td>400'000</td>
<td>Estimated to be 1 million</td>
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<td>Stability</td>
<td>24 months</td>
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<td>Formulation</td>
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<td>Dispersible tablet for all ages</td>
<td>Tablet (adult) Crushed tablet (child) Dispersible (child, 2012)</td>
<td>Tablet (adult) Crushed tablet (child)</td>
<td>No data</td>
<td>Tablets</td>
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</table>

The table includes recently launched products or products which have completed phase III clinical studies and are expected to be launched over the next 2 years. Currently, the market share for ACTs is approximately 160 million treatments, of which over 100 million are artemether–lumefantrine (Novartis plus generic versions), and most of the rest are a fixed-dose combination of artesunate-amodiaquine.

*Product developed by Sigma Tau, in collaboration with MMV.

*Drugs for Neglected Disease Initiative.

*This medicine was developed by sanofi-aventis and DNDi, and MMV is partnering with sanofi-aventis and DNDi for Phase IV studies.

*Product developed at Farmanguinos, but to be manufactured by Cipla for some markets outside Brazil; a fixed-dose combination has also been produced by Mepha, but only at premium prices.

*Coartem®D is child-friendly dispersible form of artemether–lumefantrine launched this year; the original tablet form, Coartem®, was launched in 2001.

*Dihydroartemisinin-piperaquine is sold by Chongqing Holley as Artekin®: this has not been prequalified by WHO, or approved by a Stringent Regulatory Authority.
haemolysis in G6PD-deficient patients and must be given daily for 14 days to be effective; such compliance is unachievable in the field, and some countries even have 5–7 days in their treatment guidelines (even though clinical evidence says this does not work). A third-generation 8-aminoquinoline, tafenoquine, is in clinical development, and could be given as a single dose. A phase II study to determine the safe dose of tafenoquine in G6PD patients is ongoing, sponsored by GSK. The parallel efficacy study is planned to start in 2011. The challenge in this field is the identification of new chemical entities which can kill the hypnozoite without the liabilities of the 8-aminoquinolines to G6PD patients. The most significant barrier to success here is undoubtldely that the currently available animal models cannot accurately predict the safe and the effective exposure needed in man. Mouse models of G6PD dependent haemolysis are being validated, but will still carry the considerable risk that clearance of deformed erythrocytes may be different between humans and mice. There are two additional barriers: first, it is currently impossible to maintain stable long-term cultures of P. vivax – so there are no standard ‘relapsing’ clones to study. This is important since relapse rates can be highly variable. Second, there are no cell-based models of hypnozoite formation in human cell lines. Model systems are available where the related P cynomolgi infects primary rhesus cells, which suggests that a human cell line with the right cell surface antigens or the right architecture will need to be developed (Clemens Kocken, personal communication).

Drugs targeting severe malaria: This is a challenging area for drug development. Intravenous artesunate is replacing quinine as the reference medicine, based on superior efficacy and tolerability. Until recently, the major challenge was to ensure a supply of drug manufactured to international standards of Good Manufacturing Practice (GMP), so that it can be prequalified by the WHO, allowing it to be purchased by donors. In December 2010, WHO prequalified Guilin Pharmaceuticals, supported with regulatory advice from Medicines for Malaria Venture, who can provide artesunate for injection at a price that is affordable in disease endemic countries (around $1 per 60 mg vial). Any new medicine for this indication would have to show superiority over artesunate, (or quinine if artesunate fails) which would require large clinical studies, of more than 5000 patients. In any case, the development path for a new severe malaria treatment would need to initially demonstrate rapid activity in uncomplicated malaria in man, since the animal models of severe malaria are not predictive. One interesting proposal has been for the use of a rectal artesunate formulation for the emergency treatment of severe disease. In a sub-group analysis, patients more than 6 hours from hospital showed a significant benefit to being given such a suppository. This clearly illustrates the dichotomy of severe malaria: the patients who are most at risk, are by definition the ones furthest from hospital, and so access to medicines becomes the key question. This treatment is under development by the WHO TDR, and is under regulatory discussion with the UK authorities.
1.6 Prophylaxis

Prophylaxis for pregnant women and children has been promoted in many sub-Saharan African countries. In children under five, it increases haemoglobin levels, reduces the frequency of clinical episodes and reduces overall mortality. The effects in pregnant women, particularly among primigravids (first-time pregnancy), decrease the number of premature births, with a subsequent increase in the life expectancy of the newborn. A regimen of 2 or 3 doses of sulphadoxine-pyramethamine, chosen because of its long half-life, delivered during pregnancy (IPTp) have been shown to provide significant benefit both to the mother and the foetus, and is currently implemented routinely as part of comprehensive malaria control strategies throughout most endemic countries. A similar approach has been followed with young infants (IPTi). Unfortunately, sulphadoxine-pyramethamine is being increasingly compromised by drug resistance, even for such indications. The drug development challenge for new products here, however, is enormous. Before medicines can be used for IPT, then they need to be demonstrated to be active in curing malaria. However, most countries are unhappy about the idea of having the same medicine used for prophylaxis and for treatment. Furthermore, the benefit/risk ratio in IPT will fall as the incidence of malaria falls, and so it is most unlikely that new medicines will be developed for this indication.

Antimalarial medicines are used not only for treatment but also for prophylaxis of high-risk groups. Non-immune travellers to malaria endemic areas, mostly tourists, constitute an important group. Of all the drugs developed, only pyrimethamine, proguanil and atovaquone are known to have causal prophylactic activity, and stop the infection reaching the blood stage. All other chemoprophylactics work solely as blood schizonticides. The drug of choice is GSK’s Malarone, a fixed combination of atovaquone-proguanil, which has causal prophylactic, blood schizonticidal and transmission blocking activities. Mefloquine is less expensive and has a long half-life, which provides good prophylaxis even when administered weekly, but it is associated with gastrointestinal and psychiatric side effects. Both molecules are expensive, partly because of synthetic issues, but also because, since they are used in commercial markets, there is less of a price pressure on the active pharmaceutical ingredient.

1.7 Development Challenges

The development of a medicine with two active ingredients brings special challenges. First, there is the need to select two active ingredients with different mechanisms of action, which preferably are therapeutically synergistic, without adversely affecting the uptake, distribution or safety of each other. The optimum dose for each ingredient needs to be confirmed in patients. Second, co-formulation is often a challenge, since the drugs must not interact with each other chemically. The final product has to be stable, preferably for 2–3 years with less than 5% degradation, under conditions of high temperature.
(30 ± 2 °C) and relative humidity of between (65 ± 5 and 75 ± 5%). With a cost limitation of $1 per adult treatment, formulation cannot add significantly to the expense. Third, medicines may have to be dosed and formulated differently for small children and pregnant women.

1.8 The Next Generation of Antimalarials: Developing a Target Product Profile

Successful drug discovery means starting out with a clear idea of what the final product should look like, the Target Product Profile (TPP). Since the time taken to develop and launch a medicine is over a decade, this Target Product Profile must be able to address issues in the future, and show how a new medicine would be superior over the treatment that is current, and the expected treatment paradigm in 10 to 15 years time.

The push for new medicines to drive the eradication of malaria has led to the development of the concept of SERCaP: a medicine that, with a single exposure, can produce radical cure and prophylaxis against all plasmodium species. Obviously, this is an aspirational goal, and it should always be remembered that the medicine will be a combination. A single molecule need not tick all boxes, but the more needed, the higher the cost of goods and, for example, if some only contribute to transmission-blocking, there will be ethical issues surrounding their deployment. It could be that this is a ‘goal too far’, similar to desiring a new type of transport which can travel on land, in air and under water, but with the appeal of a sports car. Alternatively, it could be seen as aspirational 30 years ago as hoping for a device that sends telephone calls, shoots and replays video clips, and acts as an airline boarding pass. These examples underline that prior to the design of a perfect medicine, we must first understand the properties of the individual components.

Four sets of properties are required in the next generation of antimalarials. Putting them together in appropriate permutations would give the ideal medicine, but they are worth pursuing initially in their own right. Details of the target product profiles have been published by Medicines for Malaria Venture.

(a) Non-artemisinin-based combination therapy (NACT) for treatment of acute uncomplicated malaria in children and adults. This must have activity against the blood parasites of the 4 key species that cause malaria in humans, including organisms resistant to existing therapies. The big debate here, of course, is whether synthetic endoperoxides will be active in areas of artemisinin resistant malaria.

(b) A single agent for the radical cure of malaria caused by Plasmodium vivax and P. ovale and thus prevent relapse. Ideally, this must have a sufficiently large safety/efficacy window to be used in patients who are G6PD deficient.
<table>
<thead>
<tr>
<th>Product For</th>
<th>Uncomplicated Malaria</th>
<th>Severe Malaria</th>
<th>Radical Cure of Relapsing Malaria</th>
<th>Intermittent Preventive Treatment (IPT)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Technical name</strong></td>
<td>Oral blood schizonticide</td>
<td>Parenteral, rapidly acting blood schizonticide</td>
<td>Anti-hypnozoite</td>
<td>Causal prophylactic – prevents the initial infection of liver and stops replication in liver and in erythrocytes</td>
</tr>
<tr>
<td><strong>Gold standard</strong></td>
<td>Artemether–lumefantrine (chloroquine for <em>P. vivax</em>)</td>
<td><em>i.v.</em> artesunate replacing <em>i.v.</em> quinine as a result of the recent clinical studies</td>
<td>Primaquine (tafenoquine)</td>
<td>Sulphadoxine-Pyremethamine</td>
</tr>
<tr>
<td><strong>Key weakness to be overcome</strong></td>
<td>Twice per day treatment; low bioavailability, with second dose after 6 h. Potential dependence on fatty foods, because of low bioavailability. Mismatch between pharmacokinetics, exposing products to resistance</td>
<td>Availability of GMP material for <em>i.v.</em> or <em>i.m.</em> (artesunate). Guilin now prequalified by WHO; other suppliers submitting in 2011</td>
<td>Compliance: current drug requires 14 days of dosing. Dose-limiting haemolysis in G6PD-deficient patients.</td>
<td>Propensity for development of resistance – a failing treatment in most countries</td>
</tr>
<tr>
<td><strong>Added value for new treatments</strong></td>
<td>New mechanism of action (no cross resistance with artemisinins or 4-aminoquinoline/amino-alcohols)</td>
<td>None – until artemisinin resistance becomes common.</td>
<td>Maintain effects against hypnozoites and gametocytes without toxicity and with 3 days of dosing.</td>
<td>Lower propensity for development of resistance.</td>
</tr>
<tr>
<td><strong>Cost</strong></td>
<td>Less than US$1</td>
<td>Less than US$5 per patient</td>
<td>Less than US$10</td>
<td>Less than US$1</td>
</tr>
</tbody>
</table>
(c) A medicine that can block the transmission of *Plasmodium* infection, preventing re-infection of mosquitoes and thus transmission to other people. There has been much debate about how, and even if, such a medicine could be developed. The best conceptual starting point here is primaquine, which has anti-gametocyte activity. It is not clear whether a ‘pure’ anti-gametocyte compound exists. Proof of concept studies (phase IIa) would probably involve volunteer infections, and clinical confirmatory trials (phase III) would use a combination with a schizonticide.

(d) Causal prophylactic activity. Clearly, for travellers and other migrant populations such as the military, this has always been an important area, and is one of the few places in malaria where there is a significant commercial market. However, this also includes new agents for intermittent preventative treatment of malaria in pregnant women (IPTp), children (IPTc) and infants (IPTi). Compounds would have to be shown to cure uncomplicated malaria before clinical studies on prophylaxis could be undertaken. It would also be important to have a long half-life (several weeks), which could be effected by slow release formulation provided the compound was potent (less than 10 mg/day).

Treatment of severe malaria is a difficult issue. A combination product is not required, but a compound with pure antimalarial activity would need to be shown to be active clinically in uncomplicated malaria. A compound with adjunctive activity would need to be shown to be active in a different disease, given the extreme vulnerability of these patients, and the fact they are generally small children.

As well as defining the clinical parameters, such as efficacy and safety as part of the target product profile, several other factors come into play which can be used to aid the early selection of potential candidates. The first is cost. Current antimalarials are available in disease endemic countries for prices of around $1.20 for an adult treatment, and as low as $0.30 for an infant dose. This is a tremendous achievement, but is still too expensive for many families, and will always require subsidy. Further reductions in cost for a combination product could come from selecting more potent compounds in man (many of the 4-aminoquinolines are used at doses of 10 mg/kg/day). Alternatively, lowering the cost of the raw materials is a possibility: recent work by Medicines for Malaria Venture and our partners has shown it is possible to significantly lower the bulk cost of some active ingredients. The second area is half-life. In the malaria field we have become used to medicines with a half-life of over 7 days, but this is far from the norm in pharmaceuticals. If a new generation of medicines with long half-lives is to emerge from research, this will be either as a result of focusing research on these classes of molecules, or working on slow-release formulations. Here, potency is an issue. Once per month, formulations have been made for other medicines, but are limited at a dose of 0.2 mg/kg/day. Third, one key factor to look at is the Parasite Reduction Rate in man. Artemisinins have a tremendous benefit for the patient in clearing parasites in as short a period as 24 hours, since they are active against all stages of the parasite.
lifecycle. New medicines with this potential are clearly at a premium. Finally, safety is a critical issue. Primaquine is not sufficiently safe to be given to the general population because of concerns about haemolysis in G6PD subjects, which make up 10–20% of the population in disease endemic countries.39 Similarly, dapsone has been withdrawn from therapy because of inadequate safety in these populations.50 Rarer adverse events may not arise until later in clinical development, and are usually hard to predict from preclinical data.

1.9 Finding New Molecules: Genes and Screens

Although artemisinin-based therapies have changed the lives of hundreds of millions of patients, the risk of resistance emerging should always be at the back of our minds. Early warnings have come from the Thai–Cambodian border.51 Before resistance can be declared clinically, 3 criteria have to be met. First, a change in the clinical outcome – this is clearly seen. Second, there needs to be confirmation that these patients had a reasonable plasma concentration of the drug, which is the case. Third, that there is a significant change in the IC50 of the parasite when studied \textit{ex vivo}. This has been so far hard to show conclusively, with very small IC50 shifts. There are suggestions that the parasite merely stays dormant for 48 hours, avoiding the drug pressure. Whatever the mechanism, the WHO is working hard to contain the outbreak in Southeast Asia, and the artemisinin combination therapies are still able to produce the required adequate clinical and parasitological response. However, given that it can take 15 years to bring a drug from discovery through to the market place, and that the success rates are not high, then it is prudent to invest heavily in finding new molecules and new scaffolds. Two approaches are being followed.

\textit{Fast followers}: the first approach is ‘fast followers’. Of the 4 clinically active families (quinine, mitochondrial inhibitors, artemisinin derivatives and anti-folates), Not sure that quinine is covered, might need a review type reference here all are being worked on, and are covered in later chapters. The anti-folate question – a pyrimethamine-type agent, which looks better than pyrimethamine \textit{and} works against all the resistant strains – is a challenge. Such molecules are being identified,52 but they face a high hurdle in development. Synthetic endoperoxides have been developed and are discussed in a later chapter, but will need to demonstrate activity against artemisinin-resistant parasites. Currently, the only generally accepted model would be changes in parasite clearance time in phase II clinical studies, so again this is a large hurdle to overcome.

\textit{New molecular targets}: in malaria drug discovery, the sequencing of the whole genomes of \textit{P. falciparum}53 and \textit{P. vivax}54 has been important in identifying the full range of potential targets against which a drug can be expected to interact. Moreover, it has provided the basis for comparisons between the gene expression patterns at different stages of the life cycle and between different parasite species. This data set allows searches for new target classes – never before pursued in drug discovery.55 It is still possible to prioritise novel targets
based on the likelihood of finding a small molecule inhibitor and based on their similarity to targets for which a drug has already been found. This opens up the possibility of ‘orthologue’ searching. Here, compounds in a company’s database are selected from medicinal chemistry programmes that were directed against a human target that is a close orthologue of the parasitic target. A small collection of compounds, including structurally related, active and inactive compounds against the human target can then be tested against the parasite enzyme. This has been successful with other parasites, with hit rates as high as 10–25% and can lead to compounds that are selective against the parasite target. However, it must be stressed that these targets are not ‘validated’ in the strictest sense of the word – that inhibitors working against the target have not been shown to work in man. This is a big risk: across all therapeutic areas, two-thirds of medicines fail the first time they are tested in man. Although there are many reasons to be more optimistic about the track record for anti-infectives, we still need to proceed cautiously. There is a concern that resistance generation for protein-based targets has historically been relatively rapid.

Cell-based screening: overall, in anti-infectives, target-based screening has not fulfilled its promise. Analysing the results of screening against validated molecular targets for antimicrobials, Payne et al. concluded that the molecular-based approach was not efficient at all. Target validation was an imperfect science, and going from enzyme hits to killing the microbe, was not simple. The membranes surrounding most microbes and the cell walls external to them prevent access in all too many cases. The recommendation was two-fold, either work on clinically validated targets or screen against the whole microorganism (Figure 1.3).

Advances in image processing and automation means that this is now possible. Over the last few years, several million compounds have been screened against the parasite in the intra-erythrocytic stages, resulting in over 20,000 compounds with activity below the micromolar level – a hit rate of 0.5%.

‘Reverse genetics-screening based on whole parasite’

**Figure 1.3** The advantages of performing the screening of compound libraries against whole parasites and identifying targets in parallel, once a chemical series has been elaborated. This process led to the extremely rapid identification and development of the spiroindolone NITD-609. From Ref 63.
Not only was this hit rate much higher than most of us predicted, but it is also much higher than that seen when screening molecular targets. This has also led to a sea change in the way drugs can be discovered. GSK, Novartis, and the academic consortium led by St Jude’s Hospital agreed to deposit their screening hits in a public domain archive. This opens up two possibilities: first, that chemists with an interest in a particular structural class or type can see what other information is available in terms of substructure analysis. Second, it helps to focus ongoing screening on truly novel scaffold types: there is little point in rescreening compound libraries with similar content. Early prioritisation of scaffolds can be based on half-life, speed of killing, dose in man and potential low cost of goods. How feasible is it to develop the compound in the absence of a target? It can be argued that the real target of many drugs is unknown and that for registration, the authorities are more interested in knowing that a molecule is effective and safe. Rottman et al. show that this can be done rapidly; their hit was optimised into a preclinical candidate in 3 years, this is turbocharged even by non-neglected disease standards. Target identification can come later, and in parallel with development. In the Rottman et al. work, the first hints of the target came from resistance studies – in this case, the identification of PfATP4, and the impact on protein synthesis.

Natural products: The Rottman paper has a final twist: although the original hit was identified in a natural product screening set, it is not a natural product, but a fully synthetic compound. Molecular hits from natural product collections have not been high, but where they occur, for example, quinine and artemisinin, they have been highly productive. Three hundred new anti-malarial compounds have been isolated from plants used in traditional medicine over the period 2005 to 2008. However, this promising number uses a cut-off of 11 micromolar in cellular assays. If the same cut-off used in pharmaceutical screening is applied, only 20 new structures pass – one thousandth the yield obtained from screening random pharmaceutical diversity. The barrier for what is defined as interesting suddenly goes up. This raises a fundamental question about natural products work in malaria. Either compounds should be exquisitely potent (single digit nanomolar hits have been seen in oncology, for example) or they should have some other major advantage. In malaria, many natural products have actually been tested out on patients, and therefore, if it could be verified in an observational study that these patients actually had malaria (rather than a non-specific fever) – then we might be on the road to another artemisinin.

Of all the natural products literature so far, only 2 clinical studies stand out. First, a traditional treatment from the Democratic Republic of Congo, PR 259 CT1, containing strictosamide, which has under gone a phase I safety study and a phase IIb controlled study in 65 patients treated for 7 days, and the patients had significant parasitaemia and fever (according to WHO research criteria). The proportion of patients cured at day 14 was 90.3% compared with amodiaquine artesunate at 96.9%. This is marginal; WHO guidelines are to look 28 days after treatment and change first-line treatment once cure rates are <90%, but it is unlikely that either a cinchona or artemesia decoction would do
better. Interestingly, the extract is active in murine models,\textsuperscript{70} but not \textit{in vitro} suggesting that deglycosylation may be required for activity. The second example is a decoction of \textit{Argemone mexicana} where 199 patients were treated with the extract containing 3 active ingredients, compared with 102 in the control arm with amodiaquine artesunate.\textsuperscript{71} Here, the median age was only 5 years (showing that any effect seen does not necessarily require the patient to have some immune protection. Again, the effect seen was borderline: 89\% success at day 28, compared with a 95\% success rate for amodiaquine-artesunate, but extremely exciting for an unpurified fraction. There may be other such studies in the literature, but these two serve to illustrate (a) that good clinical observational studies can be done, (b) natural products which are active in man can be identified and (c) that it is unlikely that these scaffolds would have been found by traditional screening routes. The important thing is now to use these molecules as the basis of medicinal chemistry programmes to make new medicines that better fit the target product profiles.

\textit{Drug repositioning:} screening of almost 2000 of the known approved drugs and molecules which were in phase II, yielded some interesting results.\textsuperscript{72} Astemizole was identified as a positive, which makes sense based on the similarity of its structure to the chloroquine series. Several other molecules from oncology were shown to be active. This raises the question that all of these molecules would have been interesting if they were already known to be inactive in man for their primary indication. Any molecule that has already been into man but failed for reasons of efficacy, and yet reached a reasonable plasma concentration would be a prime candidate for screening. There are several hundred of these compounds scattered across the pharmaceutical industry, and these will be a major focus for us over the next years.

1.10 Eradication: Moving Beyond the Erythrocytic Stages

The success of high-content screening in finding new chemotypes with activity against the erythrocyte stages suggests that this could be applied to the other stages of the parasite lifecycle. These assays exist for gametocytes for \textit{P. falciparum}; the challenge is to automate them and make them suitable for 384 well assays. For the hypnozoite stages of \textit{P. vivax}, the problem is more complex.\textsuperscript{45} Infective sporozoites are needed, which means having access to mosquito breeding facilities: new technologies for cryopreservation of sporozoites may facilitate this process.\textsuperscript{73} The cell culture challenges are more complex, as \textit{P. vivax} requires reticulocytes for culture. Hepatocytes are available but they have very low infection rates.\textsuperscript{74} Most work is still focused on primary human hepatocytes or infecting primate hepatocytes with the related species, \textit{P. cynomolgi}. The recent spotlight on eradication has served to increase the amount of funding for this area, and there are some initial results suggesting a cell-based assay for hypnozoites might be possible.

Having consistent tests for all of the stages of the parasite life cycle will be a major step forward in the agenda to find new medicines. A short-term objective
would be to test all the molecules that are currently in the malaria drug development pipeline in the available cell assays for all the significant stages of the parasite lifecycle discussed above, and generate malaria ‘life cycle fingerprints’ for each one. This will enable side-by-side comparison of molecules at a relatively early stage, and could be key for positioning the right medicines into the right early clinical assays.

1.11 The Malaria Research Pipeline

Over 90% of the malaria drug discovery projects underway target the asexual blood stages of *P. falciparum*. The first group are those which attempt to improve on drugs that have validated activity in human disease (traditionally known as ‘fast followers’). Strategies include improving inhibitors of dihydrofolate reductase to develop drugs that are active against resistant strains, and new inhibitors of mitochondrial electron transport to overcome atovaquone resistance. Here, the TPP is relatively simple and the key advantage of a new-generation inhibitor will be action against resistant strains of the parasite. Improvements in the potency of 4-aminoquinolines or amino alcohols, is another potential approach. In this case, there is the opportunity to make a radical change in the way the drug is presented (for example, combining a 4-aminoquinoline with an endoperoxide or combining an antimalarial with a resistance blocker). However, with the prospect of 5 fixed-dose artemisinin combination therapies being available to patients over the next 2 years, any new 4-aminoquinoline-containing compound must demonstrate a significant benefit in terms of cost and safety (Figure 1.4).

<table>
<thead>
<tr>
<th>Preclinical</th>
<th>Translational</th>
<th>Development</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Phase I</td>
<td>Phase IIa</td>
</tr>
<tr>
<td>GNF156</td>
<td>NITD009</td>
<td>OZ439</td>
</tr>
<tr>
<td>DSM0265</td>
<td>CDRI 97-78</td>
<td>Tafenoquine</td>
</tr>
<tr>
<td>Aminooindole Broad/Genzyme</td>
<td>EF22 Dialtor</td>
<td>Ferroquine sanofi aventis</td>
</tr>
<tr>
<td>AN3661</td>
<td>N-tert butyl acrylate Liverpool School of Tropical Hygiene/DFS</td>
<td>Fosmidomycin Clindamycin James Pharma GmbH</td>
</tr>
<tr>
<td>MK4815</td>
<td>AQ13 Imtech</td>
<td>Methylene Blue AQ Uni Heidelberg</td>
</tr>
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<td></td>
<td></td>
<td>SAR97276 sanofi aventis</td>
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<tr>
<td></td>
<td></td>
<td>Artemisone UHKST</td>
</tr>
</tbody>
</table>

Figure 1.4 Global antimalarial drug portfolio July 2011: projects which are in collaboration with MMV are in open boxes, projects with no active collaboration with MMV are shown with a dashed border.
Another strategy is to develop drugs based on new molecular-based targets, for which there are supporting data that an inhibitor will have an effect on the parasite, but as yet there is no clinical validation (Table 1.3). Here, the project in question is usually supported by strong in vivo data, such as the failure to be able to rescue a viable gene knock-out in *P. berghei* or *P. falciparum*. Such validation is always useful but is subject to the concern that confirmation is based on a negative result and that there are significant differences between *P. berghei* and *P. falciparum*. For many of these targets, medicinal chemistry is aided by high-resolution structural information. Pathways such as nucleoside biosynthesis have been highlighted by genome sequence analysis, leading to the identification of three potential drug targets. Dihydroorotate dehydrogenase (DHODH) has been targeted, as the parasite and mammalian forms differ significantly. Screening against the parasite DHODH has yielded potent and selective compounds that can now be optimised with the availability of high-resolution structural data of the enzyme–ligand complex. The power of the rational design approach has also been underlined by the design of inhibitors of adenosine deaminase. Transition state analogues of purine nucleoside phosphorylase have been shown to be active against

<table>
<thead>
<tr>
<th>Molecular Target</th>
<th>Mechanism</th>
<th>Key Objective</th>
<th>Development Stage</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dihydrofolate reductase DHODH</td>
<td>Folate Biosynthesis Pyrimidine synthesis Nucleoside synthesis</td>
<td>Overcome existing resistance Selectivity and potency in vivo</td>
<td>Preclinical</td>
<td>49,53,54</td>
</tr>
<tr>
<td>Purine nucleoside phosphorylase</td>
<td>Nucleoside synthesis</td>
<td>Clinical proof of concept</td>
<td>Preclinical</td>
<td>57</td>
</tr>
<tr>
<td>Adenosine deaminase</td>
<td>Nucleoside synthesis Mitochondrial respiration</td>
<td>Selectivity and potency in vivo</td>
<td>Preclinical</td>
<td>55,50</td>
</tr>
<tr>
<td>Cytochrome bc1 complex</td>
<td>Egress from erythrocytes Proteolysis of haemoglobin</td>
<td>Selectivity against host proteases, pharmacophores which do not cross react with host thiols</td>
<td>Discovery</td>
<td>63,62</td>
</tr>
<tr>
<td>Subtilisin-like protease Falcipains</td>
<td>Apicoplast lipid synthesis DNA replication</td>
<td>Lead discovery</td>
<td>Discovery</td>
<td>59,61</td>
</tr>
<tr>
<td>Histone deacetylase</td>
<td>Signal transduction</td>
<td>Active against existing resistance, selectivity against host target</td>
<td>Discovery</td>
<td>60</td>
</tr>
</tbody>
</table>

Table 1.3 Drug discovery projects in malaria: molecular targets.
P. falciparum. Some of these compounds are ready for testing in humans and have been shown to be safe, so it should be possible to rapidly evaluate the target in human malaria.

Another set of key pathways to be utilised as drug targets occur in the apicoplast in Plasmodium. This organelle is not found in the human host, and contains metabolic pathways and enzymes which are similar to those seen in plant choloroplasts. These pathways should offer targets that have a high degree of selectivity. The best studied is the 1-deoxy-d-xylulose 5-phosphate (DOXP) pathway for isoprenoid biosynthesis, which is the target of fosmidomycin, now in Phase II clinical trials in combination with other antimalarials. As might be expected, its safety profile is excellent, it clears parasites as rapidly as chloroquine and it shows good activity against existing drug-resistant strains but an adequate clinical and parasitological response has yet to be demonstrated in all appropriate patient populations. In addition, the apicoplast has unique fatty acid biosynthesis pathways that can be targeted suggesting that other parasite targets that are inhibited by herbicides should be considered. Unfortunately, these pathways can in some cases lead to slow parasite clearance times, with parasite death delayed until the next round of replication.

A third approach to drug discovery is to use the expertise on target families already shown to be successful in other therapeutic areas. Examples of this are signal transduction cascades, including kinases, as well as oncology targets such as histone deacetylase. Here, the challenge is to identify molecules that show sufficient discrimination against the host targets to ensure safety. Although lack of activity against human cell lines is an in vitro surrogate for safety, confirmation only comes in toxicology and clinical safety studies. Another challenge is presented by protease targets, such as the cysteine proteases, falcipain and the serine protease PfSub1, where our understanding of catalytic mechanism made it easy to find enzyme inhibitors, but which were hard to develop as drug candidates because of selectivity issues.

Most of these approaches target the erythrocyte stages of P. falciparum, although some of these drugs, if they can be delivered by intramuscular or intravenous route, rather than orally, and are fast acting, could be developed for severe malaria. Developing compounds that are active against gametocytes, sporozoites, exo-erythrocytic liver schizonts and hypnozoites is much more challenging as a reliable cellular model is needed to be able to define whether key metabolic pathways, such as mitochondrial respiration or nucleoside biosynthesis, are crucial for the reactivation of the hypnozoïte. Our lack of information in this area underlines how neglected the research into P. vivax hypnozoïtes has been.

1.12 Conclusions

Spurred by the global spread of resistance to the current standard antimalarial drugs, such as chloroquine and sulphadoxine-pyramethamine, the last 10 years have seen a massive expansion in the research and development of new
medicines to maintain and improve clinical control of malaria. Now, with the re-introduction of a malaria eradication agenda, the next 10 years are set to be even more demanding. In the short term, there should be a group of new ACTs available for use. The challenge will be to better understand how to deploy these for treatment and prophylaxis in the populations that are most at risk – small children and pregnant women. For the medium-term treatment of malaria, there is a strong clinical pipeline of new medicines, which could deliver additional Artemisinin Combination Therapies if these are required, a new, shorter-course co-packaged radical cure of \textit{P. vivax} malaria, and the first of a series of innovative non-artemisinin-based combination therapies (NACTs) to deal with artemisinin resistance, should it start to spread outside of Cambodia. For the long term treatment and eventual eradication of malaria there are further challenges, in particular, the urgent need for more new medicines to target the dormant hypnozoite stage of \textit{P. vivax}, and new products which will block the transmission of malaria by killing the gametocytes and inactivating sporozoites and exo-erythrocytic schizonts. At the same time we must maintain a watchful eye on the emergence of resistance and develop an understanding of its potential impact on priority needs for new medicines. With the successful registration of several new medicines, we at Medicines for Malaria Venture alongside our partners are confident that we can deliver what will be required of us.

Acknowledgements

Many thanks to the entire team at Medicines for Malaria Venture and the External Scientific Advisors over the years, for their help in defining the strategy for new antimalarial medicines. We apologise to those colleagues whose work was not cited for reasons of space. An enormous debt of gratitude has also to go to all the various governments, foundations and charities that have provided the finance required to operate our Product Development Partnership.

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