

# Can new treatment developments combat resistance in malaria?

Nicholas J. White

Mahidol Oxford Tropical Medicine Research Unit, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand

## 1. Background

Malaria control and elimination aspirations stand at a cross-roads. On the one hand, malaria morbidity and mortality have fallen substantially since the turn of the millennium as a result of considerable increases in global funding and the deployment of insecticide-treated bed nets and effective treatments [artemisinin combination treatments (ACTs)]. On the other hand, there has been an alarming increase in resistance both to pyrethroid insecticides in the vector mosquitos, and to artemisinin in *Plasmodium falciparum* parasites. Artemisinin resistance arose in Western Cambodia and currently extends across the Greater Mekong subregion [(GMS), from Vietnam to Myanmar] and now reaches the Eastern border of India.[1,2] Resistance to chloroquine arose in exactly this same place around 60 years ago, and spread to Africa at a cost of millions of lives. Sulfadoxine-pyrimethamine (SP) was then used to treat chloroquine-resistant *falciparum* malaria and resistance to that drug also emerged in the GMS and spread to Africa. Thus today the two main malaria control tools are threatened, and without them malaria will surely return with a vengeance as it did before from the 1970s to the 1990s as resistance to the insecticide dichlorodiphenyltrichloroethane and the anti-malarial chloroquine spread across the world. History tells us that it would be very unwise to ignore these current threats. So can new treatment developments combat resistance in malaria and put us back on track towards malaria elimination?

## 2. Antimalarial drug resistance: current situation

*Plasmodium vivax* is highly resistant to chloroquine in Indonesia and Oceania. Elsewhere low-grade chloroquine resistance is present in several *vivax* endemic areas (notably in South-East Asia and South America).[3] ACTs are uniformly

effective against blood stages of *P. vivax* everywhere except for the combination with SP to which there is resistance in many areas. *P. vivax* and *P. ovale* form dormant liver-stage parasites (hypnozoites), which cause relapses of the infection and can only be treated by primaquine of currently available antimalarials ('radical cure'). There is no convincing evidence of acquired resistance to primaquine.

*Plasmodium falciparum* cure rates following ACTs exceed 90% everywhere except in the GMS where worsening resistance to artemisinins has led to partner drug resistance (Figure 1). Five ACTs are currently recommended by the World Health Organization; artemether-lumefantrine, artesunate-amodiaquine, artesunate-mefloquine, artesunate sulfadoxine-pyrimethamine, and dihydroartemisinin-piperaquine.[4] Resistance to dihydroartemisinin-piperaquine is prevalent across Cambodia and adjacent Eastern Thailand, and resistance to artesunate-mefloquine has increased along the Thailand-Myanmar border. At the time of writing, artemether-lumefantrine is still reportedly efficacious in Laos and Myanmar. Resistance to mefloquine, lumefantrine, and piperaquine are confined to the GMS. Elsewhere ACTs containing these three drugs remain highly effective (<http://www.wwarn.org>). Widespread SP resistance across the tropics limits the use of artesunate-SP, although the combination remains effective in Central Asia where it remains first-line treatment (but not in North-East India, where efficacy is poor). Resistance to amodiaquine also limits the use of artesunate-amodiaquine although it retains excellent efficacy in West Africa.

Artemisinin resistance manifests as slow parasite clearance reflecting reduced susceptibility of the circulating ring stages of *P. falciparum*. [1] This is strongly associated with single point mutations in the Kelch protein gene located on chromosome 13 (K13).[1,4,5] More than 40 different K13 mutations have been associated with slow parasite clearance but a few (F446I, R539T, C580Y) predominate.[1,2] Some mutations confer intermediate effects (approximate e.g. E252Q, F446I) and others double the  $PC_{50}$  (e.g. R539T, C580Y, A675V).[1,2] Until now no higher levels of resistance have been demonstrated unequivocally so artemisinin-resistant infections still respond to treatment, albeit more slowly than before.[6] The reduced parasite killing means that with a 3-day treatment course there are up to a million times more parasites remaining for the partner drug to remove (Figure 1). Treatment failure rates increase, which further selects for resistance to both antimalarials.

## 3. New drugs

The antimalarial drug development pipeline has probably never looked healthier (Table 1).[7] Several of the compounds in development have new drug targets. In the past, antimalarial drugs were often introduced as monotherapies and at doses which were too low in some target groups. This encouraged the emergence of drug resistance. More effort will be invested in optimal dosing for the drugs currently in development by determining prospectively the pharmacokinetic-pharmacodynamic determinants of cure. All new drugs for uncomplicated malaria will be developed in combinations to augment efficacy and reduce the probability that resistance will emerge.[4] An aspirational goal of antimalarial drug development is the discovery of a treatment that provides 'Single Encounter Radical Cure and Prophylaxis (SERCaP)', i.e. kills all

the malaria parasites in the body in one dose.[8] This has only been achieved previously with SP (and then only for *P. falciparum* and *P. malariae*). Such a treatment would have considerable operational advantages, but the need to ensure everyone is cured may require high doses risking toxicity. It would seem preferable to aim primarily for a 3-day treatment, as is well established for ACTs and chloroquine,[4] and then evaluate the possibility of shortening the treatment course as

a secondary objective. Some of the new antimalarial drugs with established activity in clinical malaria (i.e. well into phase 2 testing) are discussed here.

### 3.1. AQ-13

Simple substitutions in the aliphatic side chain of the amino-quinolines alter their resistance profile. AQ13 is more active than chloroquine against chloroquine-resistant falciparum malaria. It is otherwise pharmacologically similar with a large apparent volume of distribution and slow elimination ( $t_{1/2\beta}$  ~14 days). It is currently undergoing phase 2 trials in Africa.

### 3.2. Ferroquine

This ferrocene–quinoline conjugate is structurally related to chloroquine (Figure 2). It is active against chloroquine-resistant *P. falciparum* and has been well tolerated and effective in combination with artesunate in phase 2 studies.[9] Ferroquine is slowly eliminated ( $t_{1/2\beta}$  16 days) and is biotransformed to an even more slowly eliminated biologically active metabolite desmethylferroquine ( $t_{1/2\beta}$  31 days).

### 3.3. DSM 265

This is a triazolopyrimidine-based inhibitor of the pyrimidine biosynthetic enzyme dihydroorotate dehydrogenase. It is active against both blood and liver stages of *P. falciparum* and is predicted to be slowly eliminated. It is now in phase 2 testing.

### 3.4. Ozonides

These are synthetic compounds built upon the peroxide pharmacophore that mediates the potent antimalarial activity of the artemisinins. Two ozonides have been developed OZ 277 (arterolane) and OZ 439 (artefenomel). Arterolane is already registered and being deployed in India in a fixed dose combination (FDC) with piperazine phosphate.[10] Artefenomel is in late phase 2 development. Both drugs are well tolerated and to date no major adverse effects have been identified. Arterolane is rapidly effective in falciparum and vivax malaria and is rapidly eliminated ( $t_{1/2\beta}$  2–4 h). Artefenomel is much more slowly eliminated ( $t_{1/2\beta}$  46–62 h) and is less rapidly acting than artemisinin derivatives.[11] The parasite clearance rate of artemisinin resistant *P. falciparum* was less affected following treatment with artefenomel. Studies are in progress to assess the effects of arterolane on artemisinin-resistant infections.

### 3.5. Cipargamin (KAE 609)

Cipargamin is a spiroindolone. It is the most advanced of several compounds in development which target plasmodial ATPase4. It is currently in phase 2 development. Cipargamin produces osmotic dysregulation and thus swelling in asexual parasites leading to the most rapid parasite clearance in both falciparum and vivax malaria of the antimalarial drugs yet tested; median  $PC_{t_{1/2}}$  <1 h.[12] The terminal elimination half-life ( $t_{1/2\beta}$ ) in malaria is ~21 h. Antimalarial activity is unaffected by resistance mechanisms to currently used anti-malarial drugs, but resistance resulting from point mutations in the gene encoding PfATPase 4 can be selected in laboratory studies.

### 3.6n KAF 156

This imidazolopiperazine represents a novel class of antimalarial compounds currently in phase 2 development. KAF 156 has both blood and liver stage activities. It produces rapid reduction in parasitemia;  $PC_{t_{1/2}}$  ~3.5h in both falciparum and vivax malaria. The  $t_{1/2\beta}$  in malaria is 40–50 h. The mode of action is not known. Antimalarial activity is unaffected by resistance mechanisms to currently used antimalarial drugs, but resistance resulting from point mutations in the gene encoding Pfcarl can be selected in laboratory studies.

#### 4. New antimalarial combinations

##### 4.1. Artesunate–pyronaridine

This new ACT is now registered in several countries and has undergone rigorous phase 4 testing. Pyronaridine is a Mannich base compound with some similarities to amodiaquine, although greater activity against multi-drug resistant *P. falciparum*. Artesunate–pyronaridine is generally well tolerated and is very effective. Concerns over the potential for hepato-toxicity with repeated dosing have receded since large phase 4 studies were completed recently.[13]

##### 4.2. Plasmodium vivax–tafenoquine

Blood stages of the other malarias affecting humans all respond well to the drugs used to treat *falciparum* malaria.[4] The only exception is sulfadoxine–pyrimethamine to which resistance in *P. vivax* has emerged in many areas. The great therapeutic challenge in *vivax* malaria is prevention of relapse (radical treatment). The only advance in the past 65 years has been the development of tafenoquine – a slowly eliminated ( $t_{1/2\beta}$  ~3 weeks) 8-aminoquinoline, which allows single dose radical cure.[14] Tafenoquine is now in late phase 3 studies. The main adverse effect of this class of drugs is oxidant haemolysis in glucose-6-phosphate dehydrogenase deficiency. Tafenoquine has been investigated extensively to find a method of deployment that mitigates this risk.

##### 4.3. Augmenting current treatment regimens

Various approaches are being tested to counter the threat of artemisinin resistance. None are readily suited to the three day optimized fixed dose blister packed medicines currently available. Simply increasing the individual doses of the antimalarial drugs is unlikely to be effective; there is no evidence that this provides additional benefit from the artemisinin component (i.e. the antimalarial effect is saturated), and it risks toxicity from the partner drug (except for lumefantrine where absorption of the current formulation is dose limited). Modeling suggests that splitting the artemisinin dose to twice daily would provide increased antimalarial effect by reducing the probability that the artemisinin component encountered refractory ring stages in synchronous infections. There is no clinical evidence that this would provide significant benefit but more information is needed. Extending the course of treatment to 5 or 7 days providing one or two additional asexual cycles of drug exposure to the artemisinin component has proved effective. [1] However, if only FDC ACTs are available it demands that the partner drug dosing is increased as well again risking reduced tolerability or toxicity. Alternatively, uncombined artemisinins need to be made available risking monotherapy if adherence is poor or drugs are misused. Longer courses of artemether–lumefantrine have been well tolerated, but more information is needed for the other drugs. Another option is to follow one 3-day ACT with another. This also covers three to four asexual cycles providing increased artemisinin effect, and it provides cross protection for the slowly eliminated partner drugs. Of course longer courses of antimalarial treatment risk reducing the generally good adherence to current regimens. In the treatment of tuberculosis and HIV infection, triple therapy is now standard, and it may well be that malaria should be managed similarly. Studies of triple ACTs (TACTs) are under way. Two slowly eliminated drugs (lumefantrine plus amodiaquine or piperazine plus mefloquine) are given together with oral artesunate or artemether in a 3-day regimen. Fortunately for both pairs, there is inverse susceptibility as greater resistance to one is associated with less resistance to the other.

#### 5. New approaches to deployment

In order to accelerate elimination of artemisinin-resistant *falciparum* malaria, an old and often controversial approach is being reactivated – mass drug administration. This currently uses dihydroartemisinin–piperaquine given in 3 day treatments three times at monthly intervals. Single low-dose primaquine (0.25 mg/kg) may be added as a gametocytocide. Dihydroartemisinin–piperaquine is particularly suitable as it is safe and well tolerated, and reliably provides at least 1-month post-treatment prophylaxis. Rising piperaquine resistance in Cambodia now threatens the effectiveness of this approach. A potentially powerful strategy to delay the emergence of antimalarial drug resistance is to deploy different anti-malarials at the same time as first-line therapies. Parasites, which develop resistance to one antimalarial soon find themselves inside a host receiving the other treatment and are eliminated. There is a strong theoretical argument to support this approach and given the problems that resistance has created for malaria control and elimination, there is much to recommend pursuing this actively.

#### 6. Conclusions/expert opinion

The new antimalarial compounds in development will be deployed in double, or preferably triple, combinations if they prove safe, well tolerated, and efficacious. The ACTs have ‘set the bar’ rather high for new drugs as they are very well tolerated and rapidly effective in simple 3 day regimens. Aiming in new drug development for comparably well tolerated and effective single dose treatments (SERCaP) might lead to the exclusion of valuable new treatments requiring longer courses of treatment – and so should be a secondary rather than a primary goal. As none of the drugs now in phase 2 will be generally available for 4 years or longer, alternative regimens of currently available antimalarials are needed to counter the threat that artemisinin and partner drug resistance in the GMS will spread elsewhere, and to ensure high cure rates are maintained until the next generation of antimalarial drugs arrive.

- 1. Ashley EA, Dhorda M, Fairhurst RM, et al.; Tracking Resistance to Artemisinin Collaboration (TRAC). Spread of artemisinin resistance in *Plasmodium falciparum* malaria. *N Engl J Med*. 2014;371:411–423.
- 2. Tun KM, Imwong M, Lwin KM, et al. Spread of artemisinin-resistant *Plasmodium falciparum* in Myanmar: a cross-sectional survey of the K13 molecular marker. *Lancet Infect Dis*. 2015;15:415–421.
- 3. Price RN, von Seidlein L, Valecha N, et al. Global extent of chloroquine-resistant *Plasmodium vivax*: a systematic review and meta-analysis. *Lancet Infect Dis*. 2014;14:982–991.
- 4. World Health Organisation. Guidelines for the treatment of malaria. Geneva: WHO;2015.
- 5. Ariey F, Witkowski B, Amaratunga C, et al. A molecular marker of artemisinin-resistant *Plasmodium falciparum* malaria. *Nature*. 2014;505:50–55.
- 6. WWARN Parasite Clearance Study Group. Baseline data of parasite clearance in patients with *falciparum* malaria treated with an artemisinin derivative: an individual patient data meta-analysis. *Malar J*. 2015;14:359
- 7. Medicines for malaria venture. [cited 2016 Feb 20]. Available from: <http://www.mmv.org/research-development/interactive-rd-portfolio>
- 8. malERA Consultative Group on Drugs. A research agenda for malaria eradication: drugs. *PLoS Med*. 2011;8:e1000402.
- 9. Held J, Supan C, Salazar CL, et al. Ferroquine and artesunate in African adults and children with *Plasmodium falciparum* malaria: a phase 2, multicentre, randomised, double-blind, dose-ranging, non-inferiority study. *Lancet Infect Dis*. 2015;15:1409–1419.
- 10. Valecha N, Savargaonkar D, Srivastava B, et al. Comparison of the safety and efficacy of fixed-dose combination of artemolane maleate and piperaquine phosphate with chloroquine in acute, uncomplicated *Plasmodium vivax* malaria: a phase III, multicentric, open-label study. *Malar J*. 2016;15:42.
- 11. Phyto AP, Jittamala P, Nosten FH, et al. Antimalarial activity of artefenomel (OZ439), a novel synthetic antimalarial endoperoxide, in patients with *Plasmodium falciparum* and *Plasmodium vivax* malaria: an open-label phase 2 trial. *Lancet Infect Dis*. 2016;16:61–69.
- 12. White NJ, Pukrittayakamee S, Phyto AP, et al. Spiroindolone KAE609 for *falciparum* and *vivax* malaria. *N Engl J Med*. 2014;371:403–410.
- 13. Sagara I, Beavogui AH, Zongo I, et al. Safety and efficacy of re-treatments with pyronaridine-artesunate in African patients with malaria: a substudy of the WANECAM randomised trial. *Lancet Infect Dis*. 2016;16:189–198.
- 14. Llanos-Cuentas A, Lacerda MV, Rueangweerayut R, et al. Tafenoquine plus chloroquine for the treatment and relapse prevention of *Plasmodium vivax* malaria (DETECTIVE): a multicentre, double-blind, randomised, phase 2b dose-selection study. *Lancet*. 2014;383:1049–1058