Chapter 1

Challenges and Perspectives in Malaria Treatment

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Over the decades, several actions and strategies in public health have been employed for control and eradication of malaria. However, malaria eradication as a goal has still not been achieved. Since 2000, significant progress has been made in the control of malaria, with a reduction in reported cases. According to the World Health Organization (WHO), between 2000 and 2015 malaria case incidence was reduced by 41% and malaria mortality rates by 62%. Ever since Malaria improvements alone was responsible to a 5% increase in life expectancy in the world and 12.3% in Africa Region [1]. Estimated deaths in 2015 were 429,000 (range 235,000 – 639,000), mainly in the African Region (92%), followed by Southwest Asia (6%) and the Eastern Mediterranean Region (2%). Among those affected, children under 5 were the most susceptible to death. Among the estimated deaths for 2015, 303,000 deaths were of children under five years of age; of these, 292,000 were in the African Region alone [1]. Despite this improvement, more than 3 billion people, almost half of the world population, are still at risk for infection and such cases occur due to a lack access to prevention, diagnosis and treatment, especially in low income countries [2]. The globally goals are to reduce malaria mortality rates by at least 90%, case incidence by at least 90% and eliminate malaria by at least 35 countries from 2015 to 2030 [3].

The reduction in malaria cases has been attributed to the use of insecticide-treated nets (ITNs) and indoor residual spraying (IRS) to eliminate the vector, quick diag-
nosis and the combination medicines [4,5]. Vector control is the main preventive measure to reduce parasite transmission, the use of ITNs and IRS being the most effective measures [1]. Chemoprophylaxis is also a preventive measure, although its use has limitations and its recommendation is restricted to specific groups such as pregnant women, infants and children up to 5 years of age living in endemic areas, as well as travelers [4]. If malaria is not diagnosed and treated rapidly, the infection can progress to its most severe form within a few days, especially the infection caused by the more aggressive parasite, *Plasmodium falciparum*. In this way, diagnosing and treating the infection at an early stage can be the difference between life and death [4]. For this, political will and increase in investments are required [6].

Despite this remarkable progress, malaria continues to have a strong impact on health, and recent reports in the literature confirm delayed responses to artemisinin derivatives in South-East Asia, Africa and South America [5,7,8], combined with decreasing efficacy of the partner drugs used in artemisinin combination therapy (ACT) [8,9]. The purpose of this chapter is to provide the reader with an up-to-date overview about aspects like malaria treatment and drug resistance, as well as perspectives on the development of new antimalarial drugs and vaccines.
Update to Current Anti-Malarial Drug Treatment

Quinine

Quinine (Figure 1) was the first specific drug for malaria infections, and is a blood schizontocidal agent. It is an alkaloid drug extracted and isolated from the bark of the cinchona tree, which was used to treat fevers during the 17th century [10]. Though efficacious, quinine has adverse side-effects, and drug-resistance was initially reported in 1910. This led to production of new synthetic quinoline analogues, such as chloroquine [11]. However, despite artemisinin-based combination therapy being the first-line treatment regimen nowadays, quinine is still used for pregnant women in their first trimester and has become re-established as a drug of choice in cases of severe malaria due to widespread emergence of chloroquine-resistant and, more recently, multiple-drug-resistant strains of malarial parasites [1].

Although quinine has been used for so long, recent studies still seek to elucidate the mechanism of action of this drug. It is known that quinine acts to prevent heme detoxification in the parasite’s digestive vacuole, like other quinolones [12,13]. According to a study carried out in recent years, quinine is localized in a non-acidic compartment within the food vacuole, possibly the same one oc-
cupied by haemozoin, thus providing the support that interferes with haemozoin production [14].

Resistance of *P. falciparum* to quinine has been strongly associated to a protein on the food vacuole membrane, encoded by the *P. falciparum* multidrug resistance (pfmdr1) gene [15-17]. This transmembrane protein (P-glycoprotein homologue) transports substrates from the parasite’s cytoplasm into the digestive vacuole. The resistance mechanisms are associated to an increase in the expression of the protein, and hence decreasing drug accumulation in the digestive vacuole by promoting hydrophobic antimalarial efflux [11]. Also, resistance to quinine has been linked to variations in ms4760 intragenic microsatellite in the pfhhe-1 gene, that encodes sodium hydrogen exchanger protein [18,19]. However, the use of these variations as genetic markers was controversial, because they appeared restricted to endemic areas from Southeast Asia or African countries [20].

The structures of quinine and its derivatives (chloroquine, amodiaquine, mefloquine, piperaquine, lumefantrine and primaquine) are shown in figure 1.
Figure 1: Chemical structures of (A) quinine, (B) chloroquine, (C) amodiaquine, (D) mefloquine, (E) piperaquine, (F) lumefantrine and (G) primaquine.

Chloroquine

Chloroquine (Figure 1) is a 4-aminoquinoline drug inspired from the structure of the quinoline ring in quinine. It was synthetized in Germany by Johann (Hans) Andersag and co-workers in 1934. Due to toxicity concerns in humans, chloroquine was effectively only introduced in 1945, during World War II. In the 1960s, chloroquine resistant *P. falciparum* strains emerged in Southeast Asia and the African continent. Then therapy had to be replaced by other drugs in those regions [11,21,22]. De-
spite the decline in its use, currently, WHO recommends chloroquine for the treatment of uncomplicated non-
*Plasmodium falciparum* malaria in regions with susceptible infections. It is used in regions in which *P. vivax* is the only endemic species and treatment failure rate at 28 day is below 10% [4].

The antimalarial activity of chloroquine, as well as quinine, is through the inhibiting hemozoin formation which results in the parasite’s death. So, chloroquine is a blood schizontocidal drug, but without action against hypnozoites during liver stage that occur with *P. vivax* [23]. Therefore, chloroquine has to be used in combination with other drugs to achieve radical cure in infections caused by these species.

As previously mentioned, due to chloroquine’s widespread use, resistance was developed in *Plasmodium* species, especially in *P. falciparum*. Studies found an association of two genes with chloroquine resistance, *P. falciparum* chloroquine resistance transporter (pfcrt) and pfmdr – 1. For pfmdr-1, mutations at codons N86Y, Y184F and D1246Y are molecular markers for these phenomena [24]. To mutation at N86Y an opposite effect in parasite susceptibility is observed between chloroquine and mefloquine: decreases of susceptibility to chloroquine and increases of susceptibility to mefloquine. The pfcrt is a member of the drug/ metabolite transporter superfamily that mediates chloroquine efflux. Mutation at K76T with other muta-
tions, such as Dd2 Southeast Asian allele (M74I; N75E; 7G8) and South American allele (C72S) are associated with drug resistance [25]. However, the chloroquine resistance might be reversed in clinical practice [26]. Several studies have shown that the efflux mechanism of this drug in the parasite can be inhibited by drugs such as antihistamines, calcium channel blockers, calmodulin inhibitors, and tricyclic antidepressants [27,28]. The therapeutic efficacy of chloroquine combined with chlorpheniramine, for example, was evaluated in children in Nigeria, and the results indicated significantly improved efficacy against chloroquine-resistant falciparum malaria [29].

**Amodiaquine**

In search of new drugs that could be effective in cases of resistance to chloroquine, other quinine analogues were developed. Amodiaquine (Figure 1) is one of these drugs, and it has been used to treat falciparum malaria since the 1940s, with a mechanism similar to chloroquine.

In 1990, its use was discontinued by a WHO recommendation after reports of agranulocytosis and hepatotoxicity cases associated with prophylaxis [30]. Amodiaquine administered orally undergoes extensive first-pass metabolism to N-desethylamodiaquine, its active metabolite which has a long half-life. Both have been shown to be bioactivated by several cytochrome P450s to protein-reactive quinoneimines, which may play a role in idiosyn-
cratic toxicity. Besides that, a low hepatic expression of the active glutathione S-transferases and NAD(P)H:quinone oxidoreductase 1 may increase the susceptibility of patients to this condition [31].

Despite these severe side effects being lower when amodiaquine is used in treatment, its clinical use is limited. WHO recommends amodiaquine combined with artesunate (artemisinin-based combination therapies – ACT) to treat uncomplicated *P. falciparum* malaria. It is recommended also for chemoprevention to children up to 6 months old in some areas with highly seasonal transmission during the season period in sub-Sahel Africa associated with sulfadoxine-pyrimethamine given monthly [4].

Resistance to amodiaquine is observed in South America, Asia, and East Africa. The mutations associated with resistance are on genes pfcrt at C72S and K76T (7G8 South American allele SVMNT) and pfmdr-1 at N86Y and D1246Y (on the pfcrt CVIET allele genetic background) [25].

**Mefloquine**

Mefloquine (Figure 1) is a 4-methanolquinoline highly effective blood schizontocidal drug with a long half-life. The parasiticidal activity is similar to quinine. It was discovered in 1978, and is widely use as monotherapy for malaria prophylaxis [22]. Nowadays, it is recommended by WHO for treatment of uncomplicated multidrug-re-
sistant *P. falciparum* malaria in combination with artesunate [4].

The first report of mefloquine resistance came from Thailand in 1982. Today, mefloquine resistance is observed in Southeast Asia, and sporadically in South America, India, and Africa. Increased copy number of pfmdr-1 increases mefloquine resistance. Also, changes in sensitivity to mefloquine have been noticed at codons C1034S and N1042D (observed outside Africa) [25].

**Piperaquine**

Piperaquine (Figure 1) is a dimer analogue of chloroquine that was introduced in the 1960s. Its activity against the parasite is also the inhibition of heme detoxification in the acidic digestive vacuole. An additional mechanism of action that has been suggested is the inhibition of haemoglobin proteolysis [32]. The currently available formulation is tablet that is used combined with dihydroartemisinin in the treatment of uncomplicated *P. falciparum* malaria, mainly in areas with chloroquine-resistant parasites [4]. Piperaquine is safer than chloroquine, and no serious side effects have been reported to this drug [26].

The rapid spread throughout Asia of *P. falciparum* resistance to artemisinin-based combination therapies includes the combination with piperaquine in Cambodia and Vietnam [4,32]. A recent study in South America associated mutation on pfcrf at codon C350R with de-
creased susceptibility to piperaquine [33]. Additionally, the increased number of pfmdr-1 copies is related to increased sensitivity to piperaquine. Mutations on pfmdr-1 at N86 plus Y184F also reduce piperaquine potency in strains expressing an Asian/African variant of the chloroquine resistance transporter pfCRT [25,34].

**Lumefantrine**

Lumefantrine (Figure 1) is a quinoline-related drug available as fixed-dose tablets combined with artemether. To reduce the selection and parasite resistance to this drug, it is not being used as monotherapy [1]. Its absorption capacity is limited, and it is recommended to take the drug together with food to improve its bioavailability [26]. Nowadays, this combination is the first-line ACT treatment of uncomplicated falciparum malaria in several countries, due to its safety and efficacy [35,36].

**Primaquine**

Primaquine (Figure 1) is an 8-aminoquinoline that was developed in 1944. Drug development occurred in response to cases of *P. vivax* relapse. Currently, it is the only drug available with action against the hypnozoites of *P. vivax*. Additionally, this drug exhibits a gametocidal and sporonticidal activity [11]. The combination of primaquine with an ACT treatment is recommended in areas of low-malaria transmission, reducing transmissibility of *P. falciparum* [4].
The mechanism of action of primaquine is not completely understood. But some mechanisms have been suggested, such as the interference on parasite DNA, the disruption of mitochondrial membrane, and through generating toxic intracellular oxidative potentials [25,37].

Despite the valuable contribution of primaquine in preventing relapse and transmission of parasites, this drug is associated with haemolytic toxicity in individuals with glucose-6-phosphate-dehydrogenase (G6PD) deficiency [11]. The dose recommended by WHO (single dose of 0.25 mg/kg bw of primaquine base) does not produce toxic effects even in patients with G6PD deficiency [1]. However, it is recommended the G6PD testing for those who need primaquine in higher doses (0,25 – 0,50 mg/kg bw dayly for 14 days) for preventing relapses in *P. vivax* or *P. ovale* infections [4].

**Artemisinin**

Artemisinin (also known as Qinghaousu) is a natural molecule, that was first isolated in 1972 by Chinese researchers from the leaves of the *Artemisia annua* L. (38-40) by studying an ancient extraction procedure described in 340 AD. This discover impacted dramatically the epidemiology of Malaria in a moment where success with chloroquine based treatment where declining. The discovery gave the Nobel Prize of 2015 to Youyou Tu [41]. It consists a sesquiterpene lactone containing an endoperoxide bridge (Figure 2), essential to pharmacologic action [42,43]. The
percent yield of the artemisinin obtained after extraction of the Artemisia plant represents between 0.01% and 0.8% of the dry weight, varying according to the plant material and growth conditions [38-40,44]. Moreover, chemical synthesis is complicated and economically unviable, these being the two limiting factors in commercialization of this drug [45-47].

Low solubility in water and oil is associated to administration being only oral, considered a disadvantage, due to the fact that this route is often impossible in patients with severe malaria [44,47,48]. Regarding the pharmacokinetic properties, artemisinin has a short half-life, reaching peak plasma concentrations within one-two hours, which may lead to recrudescence in cases where treatment is not able to eliminate all parasites, and parasites remain after treatment [38,49,50]. Artemisinin is metabolized primarily by CYP2B6 and converted into inactive metabolites, such as deoxy artemisinin and dihydroxy deoxy artemisinin [46,51].

Artemisinin is considered a blood schizonticide, containing activity in the erythrocyte phase, and consequent-ly preventing the development of pathological aggravation for acting on the young stages [48]. Yet, it is also a gametocide agent, limiting the transmission to other hosts and reducing parasitaemia more rapidly than other anti-malarials [52-54].

Despite the exact mechanism of action not having been elucidated, some mechanisms have been proposed
Several lines of evidence indicate that artemisinin interacts with the group heme, triggering iron-mediated cleavage of the endoperoxide bridge and producing carbon-centered radicals [44,47,55]. Thereafter, these radicals react with susceptible groups, alkylating proteins and compromising the micro organelles and membranes of the parasites, impairing their function [42,46,48,51,54]. As the endoperoxide bridge is essential for these actions, compounds without this structure are devoid of antimalarial activity [42,47,52].

Another significant mechanism of artemisinin is the inhibition of the PfATP6, a sarco/endoplasmic reticulum Ca$^{2+}$ ATPase (SERCA) enzyme [43,47,55]. This action increases the levels of calcium within *Plasmodium* plasma, causing its death [47,48,53].

An inherent disadvantage, resistance associated with artemisinin is described by slow parasite clearance [4,56-58]. Delayed clearance of the parasite is related to increased parasite half-life, and the parasitemia remains positive at 72 h after initial treatment [4,25,59]. A molecular marker of resistance is the increase in the number of young ring forms entering into a quiescent state after exposure to artemisinin, and rapidly returning to growth when artemisinin is eliminated [4,58]. This occurrence has been associated with mutations in the *pfk13* gene of *P. falciparum*, encoding the kelch-domain protein K13 [56]. Actually, multiple mutations were described throughout the entire K13 gene, such as the following: N458Y, Y493H, R539T, I543T, R561H, C580Y [4]. All mutations are found
in the propeller domain and showed strong regional differences [56,60,61]. Pfk13 in *Plasmodium sp.* has different biological functions, such as allowing ubiquitination and proteosomal degradation [25,44]. Based on these functions, low levels of ubiquitinated proteins and elevated basal levels of phosphatidylinositol 3 phosphate (PIP3) are found in kelch-mutated protein [44,57,60].

**Artemisinin Derivatives**

To mitigate the limitations in the pharmacokinetic properties and production of artemisinin, semisynthetic derivatives were developed, which are now available, including artemesunate and artemether. These drugs are known as first generation endoperoxides [38,40,44,46,48]. The chemical structures of artemisinin and artemisinin derivatives are shown in Figure 2. It permitted the development of artemisinin-based combination therapy (ACT), where an artemisinin derivative is used with a longer-acting antimalarial that has a different mode of action [4].

![Chemical structures of artemisinin, artesunate, and artemether](image)

**Figure 2:** Chemical structures of (A) artemisinin, (B) artemesunate and (C) artemether.
Artesunate

Artesunate (Figure 2) is hemisuccinate derivative of artemisinin [4,46,62]. This drug, due to its water solubility properties, is available in oral, rectal and parenteral formulations. Parenteral administration (intravenous or intramuscular) is recommended for the initial treatment of severe malaria, while rectal route is indicated as pre-referral treatment for severe malaria [4].

Furthermore, the advantage of this drug is the reliability and rapid pharmacokinetic profile [62-64]. After administration, artesunate is quickly converted by plasma esterases into dihydroartemisinin, which provides antimalarial activity [4,63]. Artesunate is immediately eliminated, with a half-life of 9-15 minutes for the intravenous route; 11.5-48.2 minutes for the intramuscular route; 51 minutes when the administration is rectal; and 54 minutes for the oral administration; while dihydroartemisinin is approximately 93% protein-bound, increasing its half-life independent of the route of administration [4,63].

According to the WHO, artesunate can be used for severe malaria at a dose of 2.4 mg/kg by parenteral administration, with repeat doses at 12 h and 24 h [62].

Artemether

Artemether (Figure 2) is the methyl ether derivative of artemisinin [4,65]. Unlike artesunate, artemether is water-insoluble, and the parenteral formulation is oil-based for intramuscular injection [63,65]. Artemether is ap
proximately 95% protein bound in plasma in, and is me-
tabolized in dihydroartemisinin [4,62]. The peak plasma
concentration is six hours after administration, and half-
life of this drug is 5.7 -7.0 hours [4,62,63]. Artemether is
considered an alternative for treatment of severe malaria,
and is commonly used when parenteral artesunate is not
available. WHO indicates this drug associated with lume-
fantrine [4,62,63].

**Artemisinin-based Combination Treatments (ACT)**

Despite the rapid effectiveness and good tolerance of
artemisinin and its derivatives, WHO recommends the
adoption of artemisinin-based combination therapies
(ACT) as first line treatment for uncomplicated malaria,
in order to avoid the development of resistance to these
drugs [44,48,54,61,66]. Therefore, artemisinin derivatives
are administered combined with other antimalarial drugs
to increase efficacy and safety [44,48,54,61,66]. The com-
binations currently used for malaria treatment are: artesu-
nate-amodiaquine, artesunate-mefloquine, artemether-
lumefantrine and artesunate-sulfadoxine-pyrimethamine
[44,48,54,61,66].

**Pyrimethamine**

Pyrimethamine (Figure 3) is a schizonticidal agent
and dihydrofolate reductase inhibitor (DHFR) [67,68].
Pyrimethamine competitively inhibits the enzyme dihydrofolate reductase, targeting the DHFR domain of the bifunctional thymidylate synthase enzyme, and consequently blocks the dihydrofolate synthesis. Dihydrofolate is an essential cofactor in DNA synthesis and cell multiplication [67-70]. Pyrimethamine acts mainly in the stages of asexual parasites [4].

Resistance to pyrimethamine is associated with point mutations in DHFR [67-69,71]. These mutations are related with the alteration of the following amino acids: alanine to valine-16 (A16V), cysteine to arginine-50 (C50R), asparagine to isoleucine-51 (N51I), cysteine to arginine-59 (C59R), serine to asparagine/threonine-108 (S108N/T) and isoleucine to leucine-164 (I164L) [67-69].

**Sulfadoxine**

Sulfadoxine (Figure 3) is a sulfonamide antimicrobial, which acts by inhibiting the activity of dihydropteroate synthase (DHPS), specifically 7,8-dihydro-6-hydroxymethylpterin pyrophosphokinase (PPPK) enzyme [70]. This enzyme is important to folate biosynthesis pathway [72-74]. Hence, this inhibition compromises pyrimidine synthesis and DNA replication [67,72]. This action primarily compromises the stages of asexual parasites [4].

Resistance to sulfadoxine was reported to be associated with point mutations in the DHPS genes, decreas-
Malaria

...ing the susceptibility of the parasite to this drug. The reported mutations in DHPS codons include serine to alanine-436 (S436A), alanine to glycine-581 (A581G), lysine to glutamic acid-540 (K540E), alanine to glycine-437 (A437G), and alanine to serine/threonine-613 (A613S/T) [67,68,71,75,76].

In general, due to high levels of resistance found in large parts of the world, sulfadoxine is often used in combination with pyrimethamine to treat malaria [67,69,71,77]. Sulfadoxine and pyrimethamine combination (SP) was initially included as a substitute for chloroquine, due to resistance to this drug [67,69,77]. However, SP remains a possible choice for malaria treatment due to synergistic antimalarial activity and its safety, efficacy and good tolerance [67,69,77]. This combination is indicated for intermittent preventive treatment in pregnant women and in infants [4,77].

**Proguanil**

Proguanil (Figure 3) is a biguanide compound that acts as a prodrug, producing active metabolites denominated as cycloguanil [4,78]. This active metabolite inhibits dihydrofolate reductase (DHFR) [4,26,79]. DHFR has an essential role in the folic acid cycle and hence DNA synthesis, recycling folates through the transfer of hydrogen atoms from NADPH to folate [79]. The parent compound has weak anti-Plasmodium activity, modifying mitochondrial electron transport [26].
Mutations in DHFR gene are associated with resistance to this drug, and also to pyrimethamine; however, some mutations in the DHFR gene may decrease affinity only for biguanide or for pyrimethamine \[70,80,81\]. The mutations related to proguanil are N51I, C59R and S108N \[80,81\].

**Atovaquone**

Atovaquone (Figure 3) is structurally similar to the mitochondrial protein ubiquinone, also denominated coenzyme Q \[78,82,83\]. Ubiquinone is associated with mitochondrial electron transport chain in aerobic respiration, and atovaquone acts by inhibiting this process, with the result that collapse of the mitochondrial membrane potential occurs \[78,82-84\]. Several enzymes are related to the transfer of mitochondrial electron, and consequently are inhibited, including dihydrofolate reductase, responsible for DNA synthesis \[78,82-84\].

Initially, atovaquone was used as a monotherapy; however, there are several reports of atovaquone treatment failure in antimalarial therapy, associated with resistance \[26,83\]. Mechanism of parasite resistance to atovaquone is related with a point mutation at position 2 in cytochrome b gene replacing tyrosine for serine (Y268S), or, in rare cases, asparaginase (Y268N) \[83\]. The recrudescence rates justify the combination of proguanil and atovaquone \[78,82,83\].
This combination is denominated Malarone, used as a prophylactic drug, and it is available is adult formulation (250 mg atovaquone/100 mg proguanil per tablet) and in pediatric formulation (62.5 mg atovaquone/25 mg proguanil per tablet) [83,85]. The combination of atovaquone-proguanil is synergistic, possibly due to atovaquone advancing collapse of the mitochondrial membrane potential, and inhibition of dihydrofolate reductase by cycloguanil [26,83].

Structures of pyrimethamine, sulfadoxine, proguanil and atovaquone are given in Figure 3.

![Figure 3: Chemical structures of (A) pyrimethamine, (B) sulfadoxine, (C) proguanil and (D) atovaquone.](image-url)
# Malaria

**Table 1:** Overview of current treatments recommended by World Health Organization for uncomplicated and severe malaria (Guidelines for the Treatment of Malaria - World Health Organization, 2015).

## UNCOMPROMISED MALARIA

<table>
<thead>
<tr>
<th>P. falciparum malaria</th>
<th>Treat child and adults with (except pregnant women in their first trimester) with one of the following ACTs:</th>
<th>Duration of treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>• artemether + lumefantrine</td>
<td>3 days</td>
</tr>
<tr>
<td></td>
<td>• artesunate + amodiaquine</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• artesunate + mefloquine</td>
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</tr>
<tr>
<td></td>
<td>• dyhydroartemisinin + piperaquine</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• artesunate + sulfadoxine/pyrimethamine</td>
<td></td>
</tr>
<tr>
<td></td>
<td>*In low-transmission areas, give a single dose of 0.25 mg/kg bw primaquine with ACT (except pregnant women, infant aged &lt; 6 months and women breastfeeding infants aged &lt; 6 months) to reduce transmission.</td>
<td></td>
</tr>
</tbody>
</table>

**Blood stage infection**

<table>
<thead>
<tr>
<th>Areas with chloroquine-susceptible infections</th>
<th>Treat adults and children with either an ACT (except pregnant women in their first trimester) or chloroquine 25 mg/kg (total dose).</th>
<th>3 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Areas with chloroquine-resistant infections</td>
<td>Treat adults and children with an ACT (except pregnant women in their first trimester). To prevent relapse in <em>P. vivax</em> or <em>P. ovale</em> malaria treat with:</td>
<td></td>
</tr>
<tr>
<td></td>
<td>** Primaquine (0.25-0.5 mg/kg bw daily) for children and adults (except pregnant women, infants aged &lt; 6 months, women breastfeeding infants aged &lt; 6 months, women breastfeeding older infants unless they are known no to be G6PD deficient, and people with G6PD deficiency.**</td>
<td>14 days</td>
</tr>
</tbody>
</table>

## SEVERE MALARIA

<table>
<thead>
<tr>
<th>P. falciparum, P. vivax, P. ovale, P. malariae or P. knowlesi malaria</th>
<th>Treat adults and children with (including infants, pregnant women in all trimesters and lactating women) with parenteral artesunate and continue with an oral ACT: Artesunate (intravenous or intramuscular). Oral ACT</th>
<th>Duration of treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>Once a patient has received at least 24 h of parenteral therapy and can tolerate oral therapy, complete treatment with 3 days (add single dose primaquine in areas of low transmission).</strong></td>
<td>at least 24h</td>
</tr>
<tr>
<td></td>
<td>3 days</td>
<td></td>
</tr>
</tbody>
</table>
New Strategies for Antimalarial Treatment

The looming threat of resistance to current therapies and the lack of a safe and active drug against all stages of the parasite life cycle has contributed to research efforts in development of new anti-malarial drugs. In 2007, the Bill and Melinda Gates Foundation, supported by the WHO, announced a campaign for the eradication of malaria with a new set of challenges [86]. Strategies to achieve these goals include: (a) new medicines with activity against gametocytes, blocking disease transmission; (b) medicines with efficient elimination of the hypnozoite or dormant forms in the liver, preventing relapses; (c) molecules with longer half-lives that provide increased protection against re-infection and potent prophylactic activity; and (d) medicines more potent and safe enough to be given as a single dose, reducing the duration of treatment and increasing compliance [87-89].

Over the past decade, there has been a transformation in the discovery and development of new anti-malarial drugs. Currently, the majority of projects in progress are linked to the Medicines for Malaria Venture (MMV), a public-private partnership established in 1999 as a nonprofit organization. Since its foundation, MMV and partners have developed six new medicines: Coartem® Dispersible (artemether-lumefantrine), Artesun® (artesunate injection), Eurartesim® (dihydroartemisinin-
piperaquine); Pyramax® and Pyramax® Granules (pyronaridine-artesunate) and recently SPAQ-CO™ (sulphadoxine-pyrimethamine and amodiaquine), which have received WHO prequalification [90].

To provide guidance during drug discovery and development, the MMV describes the requirements for Target Candidate Profiles (TCPs), which are: TCP-1: molecules that clear a sexual blood-stage parasitemia; TCP-3: molecules with activity against hypnozoites (mainly *P. vivax*); TCP-4: molecules with activity against hepatic schizonts; TCP-5: molecules that block transmission (targeting parasite gametocytes); and TCP-6: molecules that block transmission by targeting the insect vector (endectocides). TCP-2 nomenclature has been retired, since its characteristics of sustained anti-parasitic activity where incorporated by TCP-1. From this, two main types of Target Product Profiles (TPPs) are desired for medicines and correspond to TPP-1 and TPP-2. The first, TPP-1, refers to treatment of acute uncomplicated malaria in children or adults with a combination of two or more molecules with TCP-1 activity, plus TCP-5 for reducing transmission and TCP-3 for relapse prevention; for severe malaria, a parenteral formulation of a single fast-acting TCP-1. The second, TPP-2, relates to chemoprotection given to subjects migrating into areas of high endemicity or during epidemics; a combination of TCP-4 activity, potentially with TCP-1 support for emerging infections [91,92].
New Anti-Malarial Drug Candidates

Drug therapy remains the most effective option for treatment and prevention of malaria. Most new anti-malarial drug candidates have been discovered using high-throughput screening and molecular modelling to identify compounds that are active against the parasite [93]. As a result, new compounds are now undergoing clinical trials at Phase I, II and/or III: OZ277, OZ439, KAE609, KAF156, DSM265 and tafenoquine, as presented below.

New Generation Endoperoxide (Synthetic Ozonides)

The gold standard of malaria treatment is a fixed-dose combination of an artemisinin derivative and a 4 aminoquinoline or amino alcohol, which increases the world demand for artemisinin products and promotes significant changes in price [94]. In this context, the MMV in partnership with the University of Nebraska, the Swiss Tropical and Public Health Institute, and Monash University established a project to develop synthetic endoperoxides. The first-generation synthetic ozonide was OZ277 (or arteolane; Figure 4), that showed activity in Phase IIa trials in uncomplicated *P. falciparum* malaria [95]. However, the clinical activity was lower when compared to artesunate [96]. Despite this, Ranbaxy continued with the development and completed the Phase III study of OZ277 with piperaquine, approving its use in India [87,93,94] and
in seven African Nations [94]. The next generation candidate is OZ439 (or artefenomel; Figure 4) that has been designed to have superior pharmacokinetics to the artemisinins. A recent study indicated that the OZ439 presents excellent absorption, distribution, metabolism and excretion (ADME) properties [94]. Results from Phase I clinical trials demonstrated that OZ439 was safe and well tolerated, providing prolonged plasma exposure, indicating a better efficacy profile [98]. It has been recently advanced to Phase IIb study to investigate the efficacy of combination with piperaquine in uncomplicated \textit{P. falciparum} malaria [99].

**Spiroindolones**

KAE609 (or NITD609, cipargamin; Figure 4) is the first new class of anti-malarial, the spiroindolones. The discovery was made through screening of diverse chemical libraries using \textit{Plasmodium} whole-cell proliferation assay with cultured intra-erythrocytic parasites. This compound was developed by the Novartis Institute for Tropical Diseases in Singapore as part of a collaboration with the Swiss Tropical and Public Health Institute [100]. The compound inhibits the P-type cation-transporter ATPase 4 (PfATP4), promoting a fatal disruption in parasite sodium homeostasis [101].

In an \textit{ex vivo} assay the KAE609 was shown to be effective against \textit{P. vivax} and \textit{P. falciparum} [100]. A Phase II study was developed in Thailand involving 10 patients with uncomplicated \textit{P. vivax} malaria and 11 patients with
P. falciparum malaria. After treatment with 30 mg of KAE609 daily for three days, it was observed that parasitemia cleared rapidly. Furthermore, gametocytaemia was cleared by 8 hours post-dose, confirming its potential to block transmission [102]. Most recently, another Phase II study was published, which concluded that the rates of P. falciparum parasite clearance were dose dependent, with maximum effect at 30 mg of KAE609 [103].

**Imidazolopiperazines**

A novel class of anti-malarial, the imidazolopiperazines, is currently in clinical development for the treatment of uncomplicated malaria. KAF156 (or GNF156; Figure 4) was identified by high-throughput phenotypic screening [104] and developed by the Novartis Institute for Tropical Diseases in Singapore with the Swiss Tropical and Public Health Institute [87].

KAF156 presents parasiticidal activity against both asexual and sexual blood stages, exhibiting potential to reduce transmission and prevent re-infection [105]. It also has activity against a broad range of *Plasmodium* species, including multidrug resistant parasite strains with minimum toxicity to host-cell lines [104,106]. A Phase I study was developed with 70 healthy adult volunteers, and the results demonstrated that KAF156 was well tolerated, rapidly absorbed and had a terminal half-life of 42.5 to 70.7 hours [106]. Recently, a Phase II study was published contemplating a total of 21 adults with acute uncomplicated
malaria (11 with *P. vivax* malaria and 10 with *P. falciparum* malaria) to evaluate safety and efficacy with 3 days of treatment and 22 patients with uncomplicated *P. falciparum* malaria to access the cure rate with a single dose. The results indicated that the therapeutic responses were uniformly rapid, with an overall mean terminal half-life of 44.1±8.9 hours and cure rate of 67%. Assuming that other antimalarial drugs with short half-life usually cannot cure with a single dose it suggests that KAF156 has a clinically significant potency and will probably become used in combination therapy with good perspectives [104].

**Dihydroorotate Dehydrogenase Inhibitor**

Dihydroorotate dehydrogenase (DHODH) is an enzyme necessary for pyrimidine biosynthesis in protozoan parasites of the genus *Plasmodium*, acting as a primary source of energy for its survival. DSM265 (Figure 4) is the first DHODH inhibitor to reach clinical development for treatment of malaria. This compound was developed by high-throughput enzyme screen by University of Texas Southwestern, in collaboration with the University of Washington, Monash University, GlaxoSmithKline and MMV [107].

*In vitro* blood-stage studies demonstrated the effectiveness of DSM265 against nine strains of *P. falciparum*, including chloroquine-resistant and pyrimethamine-resistant parasites. The liver-stage activity was also evalu-
ated and the results demonstrated that DSM265 was effective for the elimination of both blood and liver replicative forms of *P. falciparum*. Moreover, DSM265 had potent *in vivo* antimalarial activity with an effective dose of 3 mg/kg/day [108].

A Phase I study was developed in Germany involving 22 healthy, malaria-naive adults. The volunteers received DSM265 or placebo before controlled human malaria infection by direct venous inoculation of *P. falciparum* sporozoites. The result observed was that a single dose of 400 mg DSM265 prevented the development of parasitaemia, confirming the potential chemoprophylactic [109]. Currently, the Phase IIa study of treating *P. falciparum* or *P. vivax* infections has been completed; however the results are not yet available [110].

**Aminoquinolines**

Tafenoquine (or WR238605; Figure 4) is a 3 phenoxy-substituted 8 aminoquinoline, and was discovered by researchers at the Walter Reed Army Institute of Research, USA, and developed by GlaxoSmithKline with support from the MMV [94]. The main objective of this research was replacing the primaquine, the only available drug for preventing relapse of malaria. It is well known that primaquine causes a significant increase in the risk of hemolysis in patients who are deficient in glucose 6-phosphate 1-dehydrogenase (G6PD) [111].
Tafenoquine possesses prophylactic, blood schizontocidal and gametocytocidal activity against rodent malaria parasites. When compared to primaquine, it is 4 to 100 times more active against *Plasmodium berghei* or *Plasmodium yoelii* [112]. Two acute cases of *P. vivax* malaria were treated with tafenoquine 800 mg over three days, when rapid parasite clearance was observed, without any relapse [113]. A clinical study with 1273 healthy Australian Defense Force personnel was realized in Bougainville and Timor-Leste for a period of at least 2 months. The volunteers received one of three tafenoquine regimens (400 mg once daily, 200 mg twice daily, 200 mg once daily) or daily primaquine (22.5 mg) plus doxycycline (100 mg) over 14 days for post-exposure prophylaxis. The results demonstrated a lower relapse rate for tafenoquine when compared to primaquine plus doxycycline [114].

In 2014, two Phase II studies were published for tafenoquine plus chloroquine, which showed that relapse prevention was more efficacious than with chloroquine alone [115,116]. Unfortunately, tafenoquine has the same G6PD deficiency liability as primaquine, but has the advantage of being a single-dose treatment [117]. The hope is that this new drug will be available to patients in 2017.
Figure 4: Chemical structures of new anti-malarial drug candidates: (A) OZ277, (B) OZ439, (C) KAE609, (D) KAF156, (E) DSM265 and (F) tafenoquine.

Malaria Vaccine

The fact that malaria occurs mainly in underdeveloped regions, mostly with poor health systems, coupled with the appearance of resistant mosquitoes and parasites, is the main obstacle in the fight against malaria. Since drug resistance is an increasingly recurrent problem, the devel-
Development of an effective vaccine that complements prevention and treatment actions becomes increasingly urgent, and is considered the most relevant tool for the prevention and reduction of transmission.

Research and development in this field has been an area of intense effort by many researchers over the past decades, presenting further progress after elucidating the life cycle of the parasite. Despite this, there is currently no effective vaccine for any of the five species of *Plasmodium* that cause malaria in humans [118].

The challenges faced in developing an effective vaccine against malaria are confronted with the complexity of the life cycle of parasites *Plasmodium spp*. The cycle comprises interactions between an invertebrate vector and vertebrate host (mammals), in addition to presenting several stages in intracellular and extracellular environments of the host. After the bite by the infected female of the mosquito Anopheles spp., the sporozoites are inoculated and migrate to the liver, invading the hepatocytes. At this stage, they initiate an asexual cycle of replication, releasing thousands of merozoites into the circulation. In the blood, the merozoites invade the erythrocytes by initiating a new cycle of replication, release and invasion. Parts of the merozoites differentiate into male and female gametocytes that can be captured by the vertebrate vector and thus complete the life cycle. Replication in the red blood cells and innate and acquired immune responses of
the host are responsible for all clinical symptoms of the disease [119].

More than 100 different antigens are expressed by *Plasmodium spp.* for the immune system at different stages of their life cycle [120], and therefore a thorough knowledge and understanding of the parasite’s life cycle is a key step in the discovery of antigens, establishing the molecular basis for a multicomponent polyvalent vaccine with a combination of candidate antigens of different stages of the life cycle [121].

The different approaches to the malaria vaccine’s development can be categorized into three main groups, representing the three stages of the life cycle in the human host: a pre-erythrocyte vaccine to prevent the entry and development of sporozoites in the liver; asexual vaccine in blood phase that prevents the disease aiming at the invasion of merozoite and intra-erythrocytic development; and the transmission blockade vaccine that targets the sexual and sporogonic phases to prevent the development of the parasite in the mosquito [121].

**Pre-Erythrocyte Malaria Vaccines**

Vaccine development is relatively diverse at each stage of the parasite’s life cycle. For *P. falciparum* alone more than 30 vaccine candidates are in advanced pre-clinical or clinical stages of evaluation [118,122]. Currently the RTS, S/AS01 (RTS, S) vaccine developed by GlaxoSmithKline (GSK) is the only vaccine that, in clinical phase III studies,
demonstrated protective effect against malaria in children [123]. Furthermore, since July 2015 the European Medicines Agency’s (EMA’s) Committee for Medicinal Products for Human Use adopted a positive scientific opinion for its use outside the European Union (EU) [124].

RTS, S/AS01 is a vaccine that targets the circumsporozoite protein of *P. falciparum*, expressed by the malaria parasite at the pre-erythrocytic stage. It comprises the hybrid polypeptide RTS (recombinant fusion protein) in which regions of the *P. falciparum* circumsporozoite protein known to induce humoral (R region) and cellular immune (T region) responses are covalently bound to the hepatitis B surface antigen (S). This RTS is expressed in *Saccharomyces cerevisiae* together with free hepatitis B surface antigen (S), to form RTS, S virus-like particles. Additionally, the formulation comprises the RTS, S with the AS01 adjuvant system [125].

In a phase III randomized controlled trial with children (aged 5 – 17 months at the time of first vaccination) and young infants (aged 6 – 12 weeks at the time of first vaccination), performed at 11 centers distributed over seven countries in sub-Saharan Africa, 8,923 children and 6,537 young infants were enrolled. All randomized children and young infants were included in the ITT population, while 6,885 (77%) children and 6,003 (92%) young infants were included in the per-protocol population. Children were followed up for a median of 48 months and
young infants for 38 months after the first dose [123]. In the results of the phase III efficacy studies for the group of children aged 5 – 17 months who received 3 doses of RTS,S at one month intervals, followed by a fourth dose 18 months later, the vaccine showed efficacy of 36.3% (95% CI: 31.8 – 40.5%) against clinical malaria. In addition, the 4-dose vaccine schedule reduced severe malaria by 31.5% in this age group, with reductions also seen in malaria hospitalizations, all-cause hospitalizations and the need for blood transfusions. In the case of the 6 – 12-week-old group, the vaccine showed an efficacy of 25.9% (95% CI: 19.9 – 31.5%) against clinical malaria, and was not considered adequate enough to justify its use in this group [123,126].

The final results of RTS,S phase III trial indicated the protection against malaria to be moderate both in extent and in duration. Thus, the vaccine has the potential to make a substantial contribution to malaria control when used together with other effective control measures, especially in areas of high transmission [123]. Several potential reasons for the lower efficacy of RTS,S include immunologic immaturity in neonates, interference from maternal antibodies, and less prior exposure to malaria [127].

Despite the EMA’s concluding that the RTS,S vaccine has an acceptable safety profile from a scientific point of view, [124] there are some issues about safety of the vaccine. Security of the vaccine is questionable due an unex-
plained excess of meningitis cases reported in the RTS, S group [123]. Since greater mortality has been reported in girls than boys for both clinical trials and experimental animal models, further rigorous studies should be conducted as to how the RTS, S vaccine is associated with greater mortality in girls [128]. Furthermore, due to its low effectiveness, this vaccine may not reach approvals worldwide. In terms of costs, this approach may be less effective if compared to the broadening of malaria treatment and vector control programs. Besides, it seems unlikely that a vaccine that only interrupts one stage of the parasite’s life cycle will be successful in preventing the entire life cycle [129].

Although the efficacy of most common vaccines must be in excess of 70 – 80%, malaria being a global health problem may justify the pursuit of such vaccines, even if the efficacy of these vaccines is only about 30 – 50% [130]. The RTS,S vaccine apparently will not meet the goal of malaria eradication by itself, and its integration into elimination strategies might be useful to improve the chances of success. Besides, the need for higher efficacy and a relatively long window of protection mandate that new generations of more efficacious vaccines must be sought.

**Asexual Blood-Stage Malaria Vaccines**

Since blood phase vaccines induce protective immunity, the addition of a blood-stage component to RTS,S as a multistage malaria vaccine is of great importance.
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It is known that a progressive development of naturally acquired immunity to the erythrocytic stage of malaria caused by *P. falciparum* occurs with age [131]. Besides, it was demonstrated that passive transfer of IgG from adult Africans long exposed to malaria could drive down erythrocytic malaria parasite levels in children with this disease [132]. Based on studies in mouse malaria parasites, many blood-stage vaccine candidates have been identified, and in some instances have also demonstrated protective immunity in non-human primates [133,134]. In general these antigens are involved in merozoite invasion of red cells, and there are a number of them which have reached human efficacy trials, [133] but results have generally been disappointing.

A blood-stage vaccine, FMP2.1/AS02A, based on apical membrane antigen-1 (AMA1) from the 3D7 strain of *P. falciparum*, tested in Malian children reported partial efficacy against clinical malaria, but only to parasites with identical AMA1 sequence to 3D7-allele-specific immune response [135]. Allelic diversity seen with AMA1 and other merozoite proteins presumably reflects the strong selective pressure of the host immune system on important parasite proteins involved in merozoite invasion of the red cell [136]. The recognition of highly conserved targets of immunity is crucial to overcome the allele-specific nature of immunity and thereby improve efficacy in clinical malaria. Evidence shows that anti-parasite immune responses can control infection against other stages as well, but
translating these experimental findings into vaccines for blood stages has been disappointing. Difficulties include the biological complexity of the organism, with an array of stage-specific genes, many of which in the erythrocytic stages are antigenically diverse. Most vaccine studies have focused on a single or a few antigens with an apparent functional role, but this is likely to be too restrictive, and broad, multi-antigen, multi-stage immune responses based on an understanding of biological responses are needed [136].

Another blood-stage vaccine candidate is PfRh5 (reticulocyte-binding protein homologue). This is a crucial and relatively conserved merozoite protein which binds to basigin receptor on the surface of erythrocytes [137]. Preclinical studies in non-human primates designed to test the immunogenicity and efficacy of PfRh5 were recently reported. Antibodies to PfRh5 displayed high levels of in vitro growth inhibitory activity (GIA) and immunization of non-human primates elicited protective immune responses against heterologous parasites. The human clinical trials are in progress [138].

**Whole Sporozoite Malaria Vaccines**

In contrast to the subunit vaccines aforementioned, a whole *P. falciparum* sporozoite vaccine is another malaria vaccine strategy. PfSPZ vaccine is a preparation of aseptic, purified, live (metabolically active), radiation-attenuated (nonreplicating), cryopreserved *P. falciparum* (Pf)
sporozoites (SPZ). The PfSPZ vaccine is manufactured by Sanaria Inc. under US FDA oversight in compliance with regulatory standards for purity, potency, safety, and consistency, and has been allowed for human administration by needle and syringe [139].

The first demonstration of 100% efficacy induced by the PfSPZ vaccine was realized in a phase I clinical trial realized in 2013 by Seder et al. The PfSPZ vaccine has proved safe and well tolerated when administered four to six times intravenously to 40 adults. Protection against infection was observed in all of the subjects who received the highest dosage of PfSPZ (6.75 × 10^5 PfSPZ), [140] and the dose threshold observed is consistent with prior studies, in which >90% protection was associated with exposure to >1000 irradiated PfSPZ infected mosquitoes [141]. Zero of six subjects receiving five doses and three of nine subjects receiving four doses of PfSPZ vaccine and five of six nonvaccinated controls developed malaria after controlled human malaria infection (CHMI) [140]. Good results from clinical studies with PfSPZ have led to an explosion of interest in whole sporozoite vaccines. However, there are hurdles yet to be overcome, including the number of doses (currently five are required), optimal dose (should the numbers of sporozoites/dose be increased), and durability of protection (whether increasing the dose will increase efficacy and especially durability of efficacy,
in malaria-endemic areas). To address these issues, Sanaria Inc. has embarked on nine further clinical trials with PfSPZ Vaccine (as registered at ClinicalTrials.gov) in malaria-endemic countries (Mali, Tanzania, Equatorial Guinea) and in the United States (NIAID, NIH; NMRC/ WRAIR) [142].

The only other malaria vaccine that induced 100% protection is the chemoattenuated vaccine (PfSPZ-CVac). PfSPZ-CVac was safe and well tolerated by 42 volunteers, and no serious adverse events were observed even upon increasing the dose. Three doses of PfSPZ-CVac ($5.12 \times 10^4$ PfSPZ) administered at 4-week intervals protected 9 out of 9 (100%) vaccinees against CHMI ten weeks after the last dose [143]. As with the PfSPZ vaccine candidate, there are still many hurdles that have to be overcome by PfSPZ-CVac.

Within the next years, it is likely that the current rapid progress will help establish whether PfSPZ-based vaccines meet the WHO malaria vaccine roadmap or US military requirements. This will depend on the observed results of protection to heterologous *P. falciparum* strains, as multiple strains normally occur in malaria-endemic areas. Besides that, alternative routes (non-IV) should be considered to facilitate administration. Once these vaccines reach licensure, plans will have to be developed for their distribution and stockpiling, and immunization of target populations, likely to be military and civilian travel-
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ers in the first instance. In the longer term, vaccination of populations at risk of malaria transmission will require strengthening of distribution centers and health-care delivery facilities capable of storing and administering these vaccines [142].

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