

# Chapter 2

## Malaria Diagnosis, Therapy, Vaccines, and Vector Control

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### 1 Introduction

Depending on the transmission intensities and the age of the patient, human malaria manifests with different clinical outcomes ranging from asymptomatic malaria, mild uncomplicated disease to life-threatening severe disease. In addition, different vectors can be associated with different parasite strains, thereby further influencing the clinical outcomes. In this context, it appears evident that diagnosis, therapy, vaccine, and vector control approaches must be multiple. The present chapter will review the current state of the art of the approaches currently employed to counteract the various manifestations of malaria.

### 2 Malaria Diagnosis

An effective management and treatment of malaria depends on early and accurate diagnosis, as misdiagnosis can result in significant morbidity and mortality. In particular, it is essential to diagnose the presence of *P. falciparum* early since the infection can be fatal within few days.

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The diagnosis of malaria is sought on clinical signs and symptoms and is based on the detection of parasites in the blood (parasitological diagnosis). The first symptoms of malaria are nonspecific (headache, muscle pains, fatigue, fever, chills, nausea, vomiting) and similar to several viral illnesses. In many endemic areas, malaria is therefore frequently overdiagnosed. World Health Organization (WHO) recommends rapid parasitological confirmation of diagnosis (by microscopy or malaria rapid diagnostic test [RDT]) in all patients with suspected malaria prior to the start of treatment (WHO 2013). This is important either to restrict use of antimalarial drugs only to the patients with malaria or to reduce the emergence and spread of drug resistance.

## 2.1 Microscopic Diagnosis

Microscopic slide examination of peripheral blood is regarded as the “gold standard” for malaria diagnosis and remains the most widely used test in most endemic areas. When used appropriately, microscopy is inexpensive, rapid, and relatively sensitive offering significant advantages over other methods. Sensitivity is about 10 parasites/ $\mu\text{L}$  and about 500 parasites/ $\mu\text{L}$  for thick film and thin film, respectively. Although thick film is more sensitive for detecting the presence of parasite, thin film can be used for accurate identification of species (except for *P. knowlesi* infection) and for counting the parasitemia. Microscopy offers indeed the advantage to identify the presence of mixed infections and can also be used to monitor the efficacy of therapy by successive examination of the parasitemia until the complete clearance (Kilian et al. 2000; Mayxay et al. 2004). However, microscopic slide examination might not be able to detect very low parasitemias; moreover, it is time consuming and needs adequately trained and skilled people while reading the smears to make accurate and reproducible diagnoses.

Thick blood films consist of a thick layer of lysed red blood cells (RBCs) since it is stained as unfixed preparation using diluted Giemsa or Wright’s stain (see Table 2.1). The thin blood film is methanol fixed, thus the morphological identification of the parasite species is much easier.

A standard way of estimating the parasite count per  $\mu\text{L}$  of blood on thick film is to use a standard value for the leucocytes count (8,000 WBC/ $\mu\text{L}$ ). Counting the number of parasites present in thick film until 200 white blood cells (WBCs) has been seen and then multiplying the counted parasites by 40 will give the number of parasite count per microliter of blood (Warhurst and Williams 1996). Quantification should also be performed using a thin film by counting the number of parasitized RBCs (not individual parasite) per 100 RBCs in a thin film (at least 1,000 RBCs have to be counted).

Different morphological characteristics of both parasites and infected RBCs should be considered for accurate species identification in a thin film (Table 2.1).

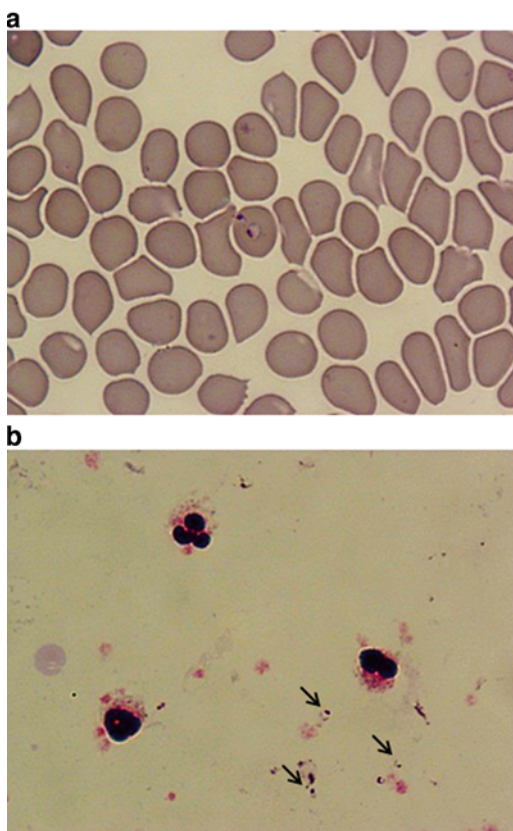
In general, all stages of the parasite can be seen in the case of *P. vivax*, *P. ovale*, and *P. malariae*, but usually only the ring parasites (see Fig. 2.1) and the banana-like

**Table 2.1** Diagnosis of *Plasmodium* species in Giemsa stained thin film

	<i>P. falciparum</i>	<i>P. vivax</i>	<i>P. malariae</i>	<i>P. ovale</i>
Ring forms	Always present in peripheral blood. Small-medium size. Multiple infection, marginal position, double chromatin dots common	Large cytoplasm, large chromatin dot. Multiple infections not common	Thick cytoplasm, large chromatin dot. Multiple infections not common	Round to oval. Thick cytoplasm, large chromatin dot. Multiple infections not common
Trophozoites Growing form	Rarely present (only in severe infections). RBCs unaltered in size. Compact cytoplasm, dark pigment	Enlarged RBCs. Large and ameboid parasite. Yellowish-brown pigment	RBCs unaltered in size. Parasite compact, occasionally band form. Dark-brown pigment	RBCs unaltered or slightly enlarged in size, some oval and fimbriated. Dark-brown pigment
Schizonts	Rarely present (only in severe infections). RBCs unaltered in size. 8–24 merozoites and dense black pigment clumped in one mass	Normally present in peripheral blood. RBCs enlarged. Large parasite. 12–24 merozoites. Yellowish-brown pigment	Normally present in peripheral blood. RBCs oval, fimbriated. 6–14 merozoites with large nuclei. Dark-brown, central pigment	Normally present in peripheral blood. RBCs unaltered in size. 6–12 merozoites with large nuclei, sometimes forming a rosette. Central mass of dark brown pigment
Gametocytes	RBCs distorted by parasite. Parasites are with crescent shape. Chromatin in a single mass in macrogametocyte or diffuse in microgametocyte	RBCs enlarged in size. Large, round parasites. Chromatin compact in macrogametocyte or diffuse in microgametocyte	RBCs normal in size. Round parasites. Chromatin compact in macrogametocyte or diffuse in microgametocyte	RBCs slightly enlarged. Round parasites. Chromatin compact in macrogametocyte or diffuse in microgametocyte
Age of infected erythrocytes	Young and old	Young	Old	Young

*RBCs* red blood cells

**Fig. 2.1** *Plasmodium falciparum*: ring stage trophozoites in a thin (a) and a thick (b) blood smear. Rings are marked with arrows (Courtesy of Dr. Romualdo Grande, Azienda Ospedaliera L. Sacco, Milan, Italy)



gametocytes are distinguished in peripheral blood in the case of *P. falciparum* malaria since mature parasites are sequestered.

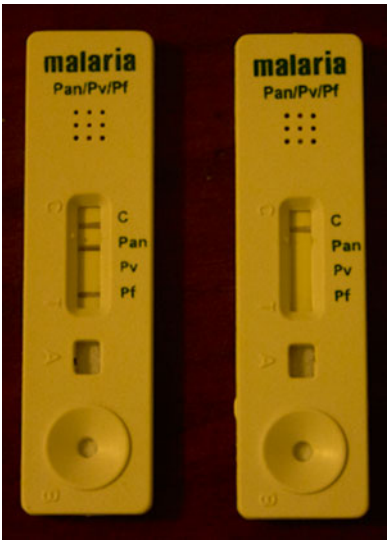
Microscopy alone is insufficient to diagnose *P. knowlesi*, forms of which can be confused with *P. malariae* and a PCR test is required to confirm the infection. With high parasitemia *P. malariae*-like parasites should be treated for *P. knowlesi* infection.

The presence of malaria pigment in leukocytes (neutrophils and monocytes) is a prognostic marker of severe malaria (Nguyen et al. 1995).

## 2.2 Rapid Diagnostic Test

Malaria RDTs were developed in the mid-1990s (Dietze et al. 1995; Palmer et al. 1998) and are based on the detection of antigens or enzymatic activities associated with the parasites. RDTs are quick (provide results within 20 min), simple, and easy to perform. A blood sample collected from the patient is placed in a test strip;

**Fig. 2.2** RDTs positive or negative for *P. falciparum*



**Table 2.2** Parasite species and target antigen of RDTs

	Target antigen		
	HRP2	pLDH	Aldolase
Species of parasites detected	<i>P. falciparum</i> specific	<i>P. falciparum</i> specific	Pan specific
		<i>P. vivax</i> specific	
		Pan specific	

Modified from Wilson (2012)

dye-labeled antibodies bind to parasite antigen, the resultant complex migrates on a nitrocellulose strip and is arrested by a capture antibody, forming a visible line. The presence of specific bands in the test card window indicates whether the patient is infected and a control line gives information on the integrity of the antibody–dye conjugate (see Fig. 2.2). RDTs are very useful in endemic areas since they are simple and quick to perform, easily transportable, and they do not require a source of electricity, thus offering a useful alternative to microscopy. Moreover, in non-endemic areas, they can be a complementary test in case of microscopy inexperience.

Some RDTs can detect only one species of malaria parasite, some other can detect more than one. The most common antigens for RDTs are *P. falciparum* histidine-rich protein-2 (*Pf*HRP2), specific for *P. falciparum*, and two enzymes of the parasite glycolytic pathways, namely plasmodial lactate dehydrogenase (pLDH) and aldolase. LDH can be specific for *P. falciparum* or *P. vivax* or it can be a variant pan specific (common to all species: *P. falciparum*, *P. vivax*, *P. malariae*, *P. ovale*, and *P. knowlesi*); aldolase is pan specific (see Table 2.2).

Another advantage in the use of RDTs is that parasite antigens, in particular *Pf*HRP2, can also be measured when mature parasites are sequestered. Sequestration

of mature trophozoites and schizonts in the microvasculature of vital organs is a central feature in the pathogenesis of *falciparum* malaria. Peripheral blood parasitemia counted by microscope does not take into account sequestered parasitized RBCs. However, these parasites secrete *Pf*HRP2 into the plasma and thus plasma concentration of this protein can provide a better estimation of the total parasite biomass (Dondorp et al. 2005b). Moreover, it is possible to measure antigens when parasite detection by microscopy is difficult because most of them are sequestered and present at very low parasitemia in the peripheral blood.

The disadvantage in the use of the RDTs is related to the multiplicity of different commercial products with different quality control and variability in the specificity and sensitivity (WHO 2011). The WHO and the Foundation for Innovative New Diagnostics (FIND) offer a testing program to evaluate the performance of RDTs (WHO 2011). Moreover, RDTs suffer of several diagnostic limitations: (1) antigens specific for *P. ovale*, *P. malariae*, or *P. knowlesi* are missing; (2) they cannot be used to determine the parasitemia or to follow the therapy since the malaria antigens (in particular *Pf*HRP2) can persist in the blood after the parasite clearance or are produced also by gametocytes, which are not usually killed by the standard antimalarial therapy; (3) persistence of the *Pf*HRP2 antigen in the blood after parasite clearance can lead to false positives (Iqbal et al. 2002; Mayxay et al. 2001; Mharakurwa and Shiff 1997; Mueller et al. 2007); (4) some isolates from the Amazon region and from Africa and India have been found lacking the *Pf*HRP2 (Gamboa et al. 2010; Houzé et al. 2011; Kumar et al. 2013), which means that RDTs based on this antigen cannot be useful in these regions.

### 2.3 New Malaria Diagnostic Targets

Some alternative malarial diagnostic targets have been explored and include proteins whose genes are highly conserved (Mouatcho and Goldring 2013).

Heat-shock proteins (HSPs) are produced at high levels by the parasite in response to increases in body temperature during malaria fever (Sharma 1992). Among HSPs, HSP-70—which is most abundant—has been explored as a new diagnostic target. Elevated levels of anti-*Pf*HSPs IgM and IgG have been found in the serum of malaria patients (Minota et al. 1988; Zhang et al. 2001). A *P. vivax* recombinant HSP-70-based ELISA has been shown to detect anti-*Pv*-HSP70 antibodies in the plasma of *P. vivax* and *P. falciparum* malaria with a sensitivity of 88.8 % and 78.8 % for *P. vivax* and *P. falciparum*, respectively (Na et al. 2007).

Another possible diagnostic target antigen could be the *Plasmodium* heme detoxification protein (HDP), a novel identified protein that binds heme and converts it into nontoxic hemozoin (Jani et al. 2008). HDP is present in all the species of *Plasmodium*, is functionally conserved, and seems to be critical for the survival of the parasite (Jani et al. 2008; Vinayak et al. 2009). Monoclonal Abs against *Pf*HDP have been developed and their specificity and affinity were comparable to

commercially available histidine-rich protein antibodies, making them promising for further test development (Kattenberg et al. 2012).

Dihydrofolate reductase (DHFR) is an enzyme of the folate pathway that catalyzes the reduction of dihydrofolate to tetrahydrofolate. Unlike bacteria and higher eukaryotes, protozoan DHFR forms a bifunctional enzyme with thymidylate synthase (TS) (Sherman 1979). Moreover, the plasmodial DHFR domain contains two additional regions whose sequences vary slightly in all plasmodia and these differences can therefore be assessed for *Plasmodium* species differentiation (Yuvaniyama et al. 2003). Monoclonal Abs against this protein have also been developed and it has been shown that they can detect *P. falciparum* and *P. vivax* (Kattenberg et al. 2012).

Glutamate-rich protein (GLURP), a highly antigenic exoantigen of *P. falciparum* whose gene is conserved in different isolates, is another possible target only suitable for *P. falciparum* diagnosis, since the gene is not present in the other species. This protein has also been considered as a malaria vaccine candidate (Jepsen et al. 2013).

An alternative approach could be to detect host markers rather than parasite proteins or metabolites.

High mobility group box 1 (HMGB1) protein is an important mediator of inflammation implicated in sepsis pathophysiology. Elevated plasma HMGB1 levels were significantly associated with severe malaria in pediatric patients with *P. falciparum* infection suggesting that elevated HMGB1 could be a prognostic marker of disease severity (Higgins et al. 2013).

Several biomarkers have been proposed for detecting malaria-associated inflammation and infection during pregnancy. *Plasmodium falciparum*-infected erythrocytes sequester in the placenta causing placental malaria, a condition associated with reduced birth weight baby and reduced chance of surviving the first year of life. Since infected erythrocytes are not observed in peripheral blood smears, women with placental malaria are misdiagnosed and thus not treated. Detection of inflammatory mediators in the peripheral blood may provide an approach to diagnose placental malaria. A study from Cameroon reported an association between plasma-soluble TNF receptor-2 levels and low birth weight babies in women infected with *P. falciparum*, suggesting that biomarkers in peripheral blood might discriminate women with poor pregnancy outcomes as a function of infection (Thévenon et al. 2010).

Another study in Tanzania showed that in women infected with *P. falciparum* the levels of IL-10 and IP-10 increased significantly while those of RANTES decreased significantly concluding that this condition might be predictive of *P. falciparum* infections (Boström et al. 2012).

## 2.4 Molecular Diagnosis

PCR-based methods to detect malaria infection were described in the early 1990s and have proven to be the most sensitive test able to identify low levels of infection, parasite species, or mixed infections (Barker et al. 1992; Snounou et al. 1993).

Some modified PCR methods, e.g., nested PCR, real-time PCR, and reverse transcription PCR, have been developed to augment the sensitivity and specificity.

Recently, the PCR method has become widely accepted for identifying *P. knowlesi* infections since the current microscopic examination may fail to distinguish *P. knowlesi* from *P. malariae*, due to their similar morphology (Oddoux et al. 2011).

However, the utility of PCR is limited by high cost, inadequate laboratory facilities, the need for trained operators, and the time to obtain results that is too long to be appropriate in establishing the diagnosis of malaria. Even in non-endemic countries, PCR is not a suitable method for routine use, but it is more useful for confirming the parasite species after the diagnosis has been established by microscopy or RDT. Moreover, PCR is useful as a research tool in clinical trials, epidemiological studies, and for detection of molecular markers of drug resistance to antimalarial drugs.

Loop-mediated isothermal amplification (LAMP) is a simple, inexpensive, specific nucleic acid amplification method, the application of which for malaria diagnosis was reported in 2006 (Poon et al. 2006). A species-specific LAMP diagnostic method for the detection of the four human species was described in 2007 (Han et al. 2007) and more recently the method has been applied to the detection of *P. knowlesi* infection (Iseki et al. 2010; Lau et al. 2011).

LAMP method can be used under field conditions since it requires simple laboratory devices both for amplification and for detection of the target gene. The whole amplification reaction takes place continuously under isothermal conditions (65 °C), thus a simple water bath can be enough. LAMP results can also be carried out with naked eye observation in the form of either visual turbidity or visual fluorescence.

Alternatively, the amplified products can also be visualized using a fluorescent intercalating dye; when SYBR green is employed, the original orange color of the dye change into green, in case of positive amplification. The use of the UV light can increase the fluorescence intensity.

### 3 Malaria Treatment

Malaria (mainly due to *P. falciparum*) can lead to fatal outcomes in only few days, thus treatment should be started as soon as possible. The main targets of current antimalarial chemotherapy are the asexual blood stages of the parasite, responsible for the malaria symptoms. However, with the new goal of malaria eradication (Khadjavi and Prato 2010; Partnership 2008), the strategy has been changed and the ideal drug should prevent both disease transmission and the relapse of dormant liver stages of *P. vivax* and *P. ovale*. Drugs able to kill both gametocytes, responsible for malaria transmission from human host to mosquito vector, and hypnozoites, responsible for *P. vivax* and *P. ovale* relapse, are indeed urgently needed. Primaquine represents the only antimalarial currently in therapeutic use able to affect both the mature stage V gametocytes and the hypnozoites. However, primaquine may cause



hemolysis in patients with glucose-6-phosphate dehydrogenase (G6PD)-deficiency and is contraindicated in pregnancy and in young children.

Another great challenge to malaria treatment is parasite resistance to almost every class of antimalarial compounds. The use of two or more drugs with different mechanism of action in combination is now recommended for the treatment of *P. falciparum* malaria to delay development of resistance.

### 3.1 Treatment of Uncomplicated Malaria

WHO recommends artemisinin-based combination therapies (ACTs) for the treatment of uncomplicated malaria caused by *P. falciparum* parasite. ACT is the combination of a fast acting artemisinin derivative (dihydroartemisinin, artesunate, artemether) and a partner drug (amodiaquine -AQ-, mefloquine, piperazine, lumefantrine) with a different mechanism of action and longer half-life than artemisinins. Almost all countries in which *falciparum* malaria is endemic have adopted ACT as the first-line treatment policy. Unfortunately, fake antimalarials are widespread in African and Asian countries compromising effectiveness and increasing the risk for emergence of resistance (White et al. 2014). Second-line treatment for uncomplicated malaria (to be used when ACT is not available) is a combination of artesunate or quinine and doxycycline, tetracycline, or clindamycin. Quinine plus clindamycin is recommended for treatment of malaria in the first trimester of pregnancy since the safety of artemisinin derivatives during this period is not yet established (WHO 2010).

Chloroquine (CQ) in combination with primaquine for radical cure is still the drugs of choice to treat *P. vivax* malaria in endemic areas where CQ is already effective. However, ACTs should be used in areas where CQ-resistant *P. vivax* parasite has been identified.

### 3.2 Treatment of Severe Malaria

Severe malaria is a life-threatening disease and should be promptly treated with parenteral antimalarial therapy. Severe malaria is most commonly caused by infection with *P. falciparum*, although *P. vivax* and *P. knowlesi* can also be responsible (Antinori et al. 2013; Price et al. 2009). Over the years, quinine has been the mainstay in the treatment of severe malaria. More recently, injectable artesunate (intramuscular or intravenous) has become the recommended treatment of choice for severe malaria worldwide (WHO 2010), including severe infection caused by *P. vivax* and *P. knowlesi* (Barber et al. 2013). Two large multicenter clinical trials (the first on adult patients in Asia and the second on children in Africa) compared parenteral treatment of artesunate versus quinine in patients with severe malaria revealing that artesunate treatment reduces both adult and child mortality rates (Dondorp et al. 2005a, 2010).

### 3.3 Antimalarial Drugs

#### 4-Aminoquinolines

CQ, a 4-amino 7-chloro quinoline, is the lead compound of the 4-aminoquinolines family (Fig. 2.3). It was first synthesized in 1934 and for several decades it was the most widely used antimalarial drug. The success of this drug is due to high efficacy, low toxicity, and low-cost synthesis. However, uncontrolled, massive, and often improper treatment with CQ potentiated the emergence and spread of parasite resistance. Nowadays, the use of this drug to treat *falciparum* malaria is limited to few areas even though it still maintains considerable efficacy for the treatment of *P. vivax*, *P. ovale*, and *P. malariae* infections (WHO 2010).

The mechanism of action of CQ is not completely understood. CQ and related 4-aminoquinolines are weak bases which diffuse passively through membranes along pH gradients as unprotonated molecules, and accumulate intracellularly where they are trapped in acidic compartments, become protonated, and raise the local pH (O'Neill et al. 2006). In *Plasmodia*, they accumulate in the parasite food vacuole, where host hemoglobin is degraded to peptides and free heme, which is in turn detoxified by forming inert hemozoin. The antimalarial activity of 4-aminoquinolines is ascribed to their ability to form drug-heme adducts and

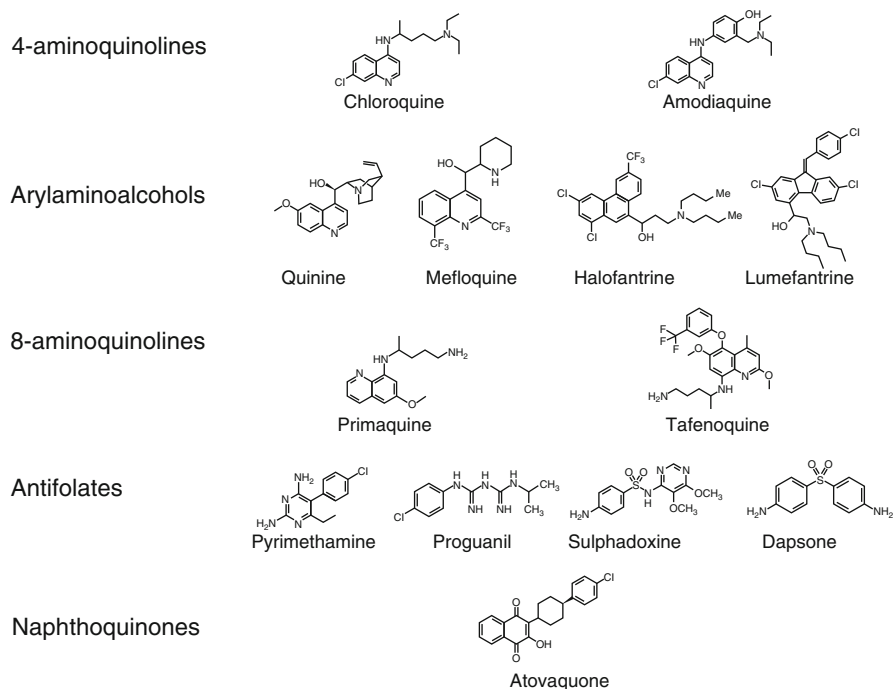


Fig. 2.3 Chemical structures of some current antimalarial drugs

accumulation of free heme, which is toxic for the parasite (Egan 2008; Ridley et al. 1997). Additional effects on parasites have also been reported, such as the inhibition of hemoglobin endocytosis and digestion or disruption of normal vesicle trafficking (Fitch et al. 2003; Hoppe et al. 2004; Roberts et al. 2008).

CQ resistance is related to point mutations in the gene encoding for the *P. falciparum* chloroquine resistance transporter (*PfCRT*) protein, resulting in reduced CQ accumulation in the food vacuole (Bray et al. 2005; Wellems and Plowe 2001).

AQ is a phenyl-substituted analog of CQ (see Fig. 2.1) with a mechanism of action similar to CQ, but still divisive (O'Neill et al. 1998). Although cross-resistance of CQ and AQ has been documented, AQ is effective against several CQ-resistant strains. However, its clinical use has been severely restricted because of hepatotoxicity and agranulocytosis.

Piperaquine is a bisquinoline antimalarial, first synthesized in the 1960s, with an excellent activity on CQ-resistant parasites (Raynes 1999; Vennerstrom et al. 1992). The exact mechanism of action of piperaquine is unknown hitherto. Nonetheless, it seems to act in a similar way to other quinolines, interfering with the heme detoxification process (Davis et al. 2005). Piperaquine has been used extensively in China as mass prophylaxis and treatment until the emergence of resistance, which diminished its use by the late 1980s. Subsequently, due to its good tolerability, pharmacokinetic profile, and low cost, piperaquine was rediscovered as a promising partner drug for ACT. A fixed oral dose combination of piperaquine and dihydroartemisinin (Eurartesim®) is now available for the treatment of uncomplicated *falciparum* malaria in adults and children.

## Aryl Amino Alcohol

Quinine, a member of the cinchona alkaloid family (Fig. 2.3), is one of the oldest antimalarial agents. Cinchona bark extracts were identified as early as 1600 to be effective in treating the tertian fever of malaria. In 1820, quinine was isolated and named by Pierre Joseph Pelletier and Joseph Caventou and remained the mainstay of malaria treatment until the 1920s, when more effective synthetic antimalarials, such as CQ, became available. Quinine was successfully used in mass campaigns that, together with other control measures, led to the eradication of malaria in Italy at the start of the twentieth century (Brown 1997).

Quinine has rapid schizonticidal activity against intraerythrocytic malaria parasites. Its mechanism of action has not been fully elucidated. As the quinoline antimalarials, the most widely accepted hypothesis is that the drug can inhibit hemozoin crystallization interfering with the heme detoxification process.

Although the drug has been used since the seventeenth century, resistance was first reported in 1910 after hundreds of years of use, differently from the resistance to CQ that emerged within only 12 years of its introduction (Peters 1982). Moreover, resistance to quinine is usually in low grade. It has been well documented in Asia (Mayxay et al. 2007) and South America (Legrand et al. 2008), whereas conflicting results have been reported in Africa (Mutanda 1999; Pradines et al. 1998;

Tinto et al. 2006; Touré et al. 2008). Documentation of quinine resistance has been decreasing in this century due to the increasing use of artemisinin.

Quinine has been the mainstay for treating severe malaria since the results of the SEAQUAMAT and AQUAMAT trials have shown the superiority of artesunate with respect to quinine (Dondorp et al. 2005a, 2010). Quinine is now a second line of choice when artesunate is not available.

Mefloquine, a 4-methanolquinoline (Fig. 2.3), was developed at the Walter Reed Army Institute of Research in the 1970s in response to concerns regarding the increase of CQ-resistant parasite. Mefloquine is a blood schizonticide, active against the erythrocytic stages of all malaria species that infect humans, including *P. knowlesi* (Bronner et al. 2009). Mefloquine, atovaquone/proguanil, and doxycycline are all medications licensed and recommended for malaria chemoprophylaxis to areas of chloroquine-resistant *P. falciparum*. Mefloquine is indeed effective in the prevention of malaria, except in clearly defined Thai border regions of multidrug resistance. The mechanism of action of mefloquine is still unknown, probably being different from 4-aminoquinolines. Activity on the parasite seems to be related to the ability of mefloquine to interfere with the transport of hemoglobin from the erythrocyte to the food vacuole (Olliaro 2001).

Halofantrine and lumefantrine are other two compounds of this class, on the market alone or in combination with an artemisinin derivative, respectively. Lumefantrine is available only in an oral preparation co-formulated with artemether.

## 8-Aminoquinolines

8-Aminoquinolines (Fig. 2.3) are an important class of antimalarials because they belong to the only class proven to be effective against the hypnozoites of *P. vivax* and *P. ovale*. Primaquine is certainly used to achieve radical cure (complete elimination) of relapsing malaria due to *P. vivax* or *P. ovale*, in combination with a blood schizonticide for the erythrocytic parasites. In addition to the activity against hypnozoites, 8-aminoquinolines can kill gametocytes and consequently block the malaria transmission. The addition of a single dose of primaquine to ACT treatment is, therefore, recommended by WHO to reduce gametocyte burden and thus transmission. G6PD deficiency should be evaluated before the administration of primaquine since the most significant adverse effect is hemolytic anemia in patients with G6PD deficiency. Primaquine is not recommended for pregnant women and for children under 4 years (WHO 2010). The mechanism of action of primaquine is unknown but the mitochondria may be the biological target. It is thought to interfere with the cellular respiration of the parasite by means of generating oxygen-free radicals and deregulating the electron transport (Krungskrai et al. 1999).

Tafenoquine is a primaquine analog developed at the Walter Reed Army Institute of Research in 1979 with aim to find less toxic and longer acting 8-aminoquinolines. Tafenoquine possesses higher activity than primaquine both in in vitro assays on *P. falciparum* and in in vivo animal models with *P. berghei* (Coleman et al. 1992;

Vennerstrom et al. 1999). Moreover tafenoquine retains gametocytocidal and sporontocidal activities representing a hopeful candidate agent for transmission-blocking public health applications.

## Antifolates

Antifolates (see Fig. 2.3) are drugs able to block the synthesis or the conversion of folate derivatives, thus indirectly they have an effect on the synthesis of nucleic acids in the malaria parasite.

The antifolates currently in use can target two important enzymes of the folate pathway, namely the DHFR and dihydropteroate synthase (DHPS). Pyrimethamine and proguanil target DHFR, whereas sulfadoxine and dapsone act on DHPS. These drugs have not been used for long time alone since it was demonstrated that DHPS inhibitors could synergize with inhibitors of DHFR (Bushby 1969) leading to their use in combination. Proguanil, the first antifolate to be discovered, is converted in the liver into the active metabolite, cycloguanil. It is not actually used alone as resistance to proguanil develops very quickly. The combination of proguanil and atovaquone, an inhibitor of the electron transport, is known as Malarone® and it is used for both treatment and prophylaxis.

Pyrimethamine is used in synergistic combination with sulfadoxine (Fansidar®) or sulfalene (Metakelfin®) for treatment of uncomplicated malaria and with dapsone for prophylaxis.

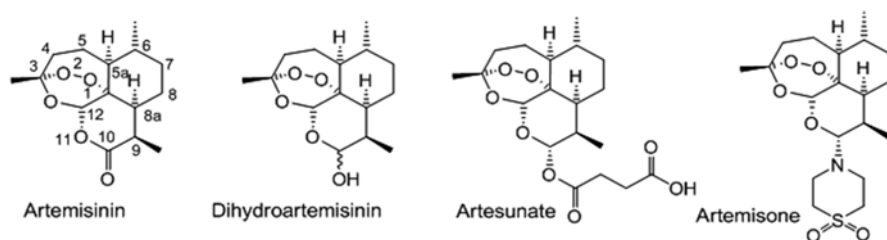
Pyrimethamine/sulfadoxine has been the drug of choice for intermittent preventive treatment (IPT) in pregnant women living in malaria-endemic areas. It has been shown that the administration of one dose of pyrimethamine/sulfadoxine in the second and third trimester of pregnancy is associated with a reduction of placental parasitemia, maternal anemia, and low birth weight (Grobusch et al. 2007).

Resistance, caused by point mutations in DHFR and DHPS, arises rather rapidly in response to drug pressure and is now common worldwide.

Atovaquone is a hydroxynaphthoquinone antiparasitic drug active against all *Plasmodium* species. Atovaquone selectively inhibits the parasite mitochondrial electron transport chain at the cytochrome bc1 complex. Selectivity is due to structural differences between the cytochrome b encoded by the parasite mitochondrial DNA and that encoded by the host mitochondrial DNA (Vaidya et al. 1993). As stated previously, it is currently used in combination with proguanil for both treatment and prophylaxis. Resistance is related to mutations of cytochrome b gene (Srivastava et al. 1999).

## Artemisinin and Derivatives

Artemisinin (qinghaosu) is an endoperoxide sesquiterpene lactone (see Fig. 2.4) extracted from the Chinese herb *qinghao* (*Artemisia annua*), a herbal remedy used in China for the treatment of fever for about 2,000 years. In 1967, the Chinese



**Fig. 2.4** Chemical structures of artemisinin and some first generation (dihydroartemisinin and artesunate) and second generation (artemisone) derivatives

government launched a coordinated program to discover antimalarial compounds in indigenous plants used in traditional Chinese medicine which led to the discovery of quinghaosu (now called artemisinin), an extract of quinghao, with potent antimalarial activity in 1971. Artemisinin is a potent and fast acting blood schizontocide killing all parasite stages including young *P. falciparum* gametocytes (stages I–IV). However, artemisinin has some pharmacokinetic limitations such as low solubility, poor bioavailability, and short half-life in vivo. To overcome some of these problems, semisynthetic derivatives have been developed. First generation derivatives include dihydroartemisinin, artesunate, arteether, and artemether. All of these compounds share the same basic chemical structure of artemisinin but possess different chemical groups at the C10 position (see Fig. 2.4). Moreover, all active compounds possess a distinctive 1,2,4-trioxane pharmacophore, which is essential for the antimalarial activity since the corresponding acyclic compounds lacking the endoperoxide are biologically inactive (Posner et al. 1992). Second generation artemisinin includes artemisone which has shown improved pharmacokinetic properties such as longer half-life and lower toxicity (Haynes 2006).

Artemisinin derivatives, alone or in combination, are the treatment of choice for severe and uncomplicated malaria, respectively. In particular, artesunate is worldwide recommended for severe malaria, including severe *vivax* malaria and *knowlesi* malaria (Barber et al. 2013).

ACT consists of an artemisinin derivative combined with a long-acting antimalarial drug. Different ACT options are now available and include artemether/lumefantrine, artesunate/AQ, artesunate/mefloquine, artesunate/sulfadoxine-pyrimethamine, and dihydroartemisinin/piperaquine. To promote patient adherence to treatment and to avoid the use of artemisinins as monotherapies, fixed-dose combination formulations into a single tablet are strongly recommended (WHO 2010) and are now available for all recommended ACTs, except for artesunate plus SP (WHO 2010).

Artemisinins should be avoided in the first trimester of pregnancy until more information is available. Morphological abnormalities in early pregnancy have been indeed demonstrated in animal studies (Longo et al. 2006). The precise mechanism of action of artemisinin is unclear and still controversial (O'Neill et al. 2010). It has been suggested that the endoperoxide bond undergoes reductive activation by

iron(II) or iron(II)-heme. This redox reaction produces carbon-centered radicals that alkylate target molecules leading to parasite's death (Meshnick et al. 1991; Olliaro et al. 2001). Alternative views suggest that artemisinin inhibits a *P. falciparum* sarcoendoplasmic reticulum  $\text{Ca}^{2+}$ -ATPase (SERCA) homolog (Eckstein-Ludwig et al. 2003). Another proposed mechanism is that artemisinins act as oxidant drugs through oxidation of FADH2 and parasite flavoenzymes (Haynes et al. 2010, 2012).

Parasite resistance to artemisinin has so far been described in Southeast Asian countries (Cambodia, Myanmar, Thailand, and Viet Nam). Several factors, such as low patient adherence to antimalarial regimens, counterfeit drugs, and monotherapies, contributed to the emergence of artemisinin resistance. If resistances were to spread, the public health consequences could be disastrous considering that no alternative antimalarials, with comparable efficacy to artemisinins, are presently available.

## 4 Malaria Vaccines

The emergence and spread of artemisinin and insecticide resistance have shown the limitations of the current recommended malaria control measures, thus a safe and protective vaccine is needed to achieve the goals of WHO's malaria eradication agenda. If malaria eradication has to be achieved, not only *P. falciparum* but also *P. vivax* vaccine has to be developed.

The rationale for a malaria vaccine development is based on the observation that people living in endemic areas develop clinical protective immunity that limits the severity of the disease and prevent mortality (Greenwood 2005).

Despite decades of intense research in this field, no licensed malaria vaccines are available until now. The most advanced one in development is the RTS,S subunit vaccine which targets the circumsporozoite protein of *P. falciparum*. The listless development in this field and the complexity in achieving a vaccine are due to the strong ability of malaria parasite to evade host's immune system. During the multi-stage life cycle, malaria parasites express different antigens, each stimulating a specific immune response. Moreover, parasites exhibit wide genetic diversity, particularly in the surface proteins which could be employed as vaccine antigens; some blood-stage antigens, such as *P. falciparum* erythrocyte membrane protein-1 (PfEMP1), show temporal switching of variant expression. There is also a lack of understanding of the specific immune responses able to confer protection against the parasite in humans.

The main required characteristics of an ideal malaria vaccine should include high efficacy in preventing clinical disease and transmission, good safety profile for young infants and pregnant women, and protection against the five species of malaria parasite or at least against *P. falciparum* and *P. vivax*.

Vaccination against malaria can target different life cycle stages. Vaccines can be indeed divided into three types:

- Preerythrocytic vaccines
- Blood-stage vaccines
- Transmission-blocking vaccines

#### **4.1 Preerythrocytic Vaccines**

Target of these vaccines are sporozoites and/or hepatic stages of the parasite. These vaccines should be able to elicit an immune response mediated by an antibody that prevents invasion or by T cells that attack the infected liver cell.

Sterile immunization with whole irradiated sporozoites, inoculated by mosquito bite, was demonstrated both in mice (Nussenzweig et al. 1967) and in humans (Clyde et al. 1973) more than 40 years ago. However, in order to obtain a whole parasite vaccine, sporozoites have to be cryopreserved and then administered via standard methods, such as intramuscular and intradermal injections. Little progress in improving these models has been made until recently. In 2011, Epstein et al. demonstrated that intradermal inoculation of cryopreserved irradiated *P. falciparum* sporozoites to 80 volunteers was safe but neither immunogenic nor protective (Epstein et al. 2011). In contrast five intravenous inoculations of attenuated, cryopreserved *P. falciparum* sporozoites protected all the six participants of the study (Seder et al. 2013). A genetically attenuated parasite has been proposed as an alternative to irradiation: by dual gene deletions, an attenuated *P. falciparum* unable to complete liver stage development has been obtained (VanBuskirk et al. 2009).

The circumsporozoite antigen was identified as the major component of the sporozoite surface and the gene was cloned and sequenced. This antigen reacts with antibodies that inhibit the invasion of hepatocytes by sporozoites and induce T cell responses against sporozoite-infected liver cells (Nardin and Nussenzweig 1993; Rieckmann et al. 1974). These findings led to circumsporozoite antigen development as a candidate vaccine and the RTS,S is now the leading candidate vaccine for malaria. RTS,S is a recombinant subunit vaccine composed of the central repeat region of *P. falciparum* circumsporozoite protein fused to hepatitis B surface antigen (HBsAg). The fused protein is coexpressed in yeast cells with free HBsAg (Gordon et al. 1995). RTS,S antigen construct on its own has limited immunogenicity and it has since been formulated with potent adjuvant system, AS02 and AS01. The results from a phase III trial in children 5–17 months of age showed reduced episodes of both clinical and severe malaria by approximately 50 % (Agnandji et al. 2011). Another large multicenter phase III trial of RTS,S in infants aged 6–12 weeks showed good safety, but moderate efficacy with 30 % protection against clinical malaria and 26 % against severe malaria for 12 months follow-up after the last vaccination (Agnandji et al. 2012). These results show that the vaccine does not meet the ambitious 2015 goal of the Malaria Vaccine Technology Roadmap that a malaria vaccine must provide 50 % protection against severe disease and death for at least 1 year.



## 4.2 Blood Stage Vaccines

The objectives of these vaccines are preventing the erythrocyte invasion and blocking the adherence of parasitized RBCs to several tissues. They do not prevent infection but attenuate the clinical symptoms of malaria. Among the large number of asexual blood stage vaccine candidates, most target merozoite antigens, such as the merozoite surface protein 1 (MSP1), 2 (MSP2), 3 (MSP3), the apical membrane protein (AMA1), the GLURP, and the erythrocyte-binding antigen 175 (EBA175). None have resulted in clear clinical protection, and generally phase II trials showed limited efficacy (Fowkes et al. 2010; Goodman and Draper 2010; Richards and Beeson 2009).

Antigens expressed on the surface of infected RBCs are highly polymorphic and thus they are not good candidates for vaccine development. An exception is a variant of the erythrocyte membrane protein 1 (*PfEMP1*) known as VAR2CSA, studied in the search of a vaccine for prevention of pregnancy-associated malaria. This is a variation of *falciparum* malaria in prima gravidas, associated with maternal anemia and placental malaria infection, reducing the birth weight and increasing the risk of neonatal mortality. Pregnancy-associated malaria is characterized by accumulation of infected erythrocytes in the placenta, and this process is mediated by parasite-encoded VAR2CSA binding to chondroitin sulfate A (CSA). The feasibility of a vaccine able to prevent complication during pregnancy is supported by the fact that women acquire immunity after one pregnancy through the production of antibodies against VAR2CSA that inhibits the adhesion of infected erythrocytes to placental CSA (Rogerson et al. 2007). Multigravidae who have acquired antibodies against VAR2CSA are indeed protected from pregnancy-associated malaria. Vaccine candidates directed against VAR2CSA are currently under development (Hviid 2010).

## 4.3 Transmission-Blocking Vaccine

Transmission-blocking vaccines target the sexual stages of the parasite preventing malaria transmission and thus they will not protect the vaccinated individual but the community by reducing the incidence of infection.

The feasibility of a transmission-blocking vaccine is supported by the observation that sera from immune individuals can block the fertilization of gametes and prevent further parasite development (Greenwood 2005; Greenwood et al. 2008). Oocyst formation and sporogonic cycle in the mosquito midgut can be blocked by specific host antibodies, complement proteins, and cytokines (Sinden 2010; Sutherland 2009). Prevention of sporogonic development can block the transmission of the parasite to the next human and subsequent spread of parasites in endemic populations. Targets of transmission-blocking immunity are surface proteins expressed on gametocytes, gametes, zygotes, and ookinetes. *Pfs25*, *Pfs28*, *Pfs48/45*, and *Pfs230* of *P. falciparum* have been shown to induce antibodies with transmission-blocking activity when ingested by the mosquito vector during the blood meal (Pradel 2007).

Transmission-blocking vaccine candidates identified in *Plasmodium vivax* are the ookinete surface proteins *Pvs25* and *Pvs28* and the gamete surface protein *Pvs230* (Sattabongkot et al. 2003; Tachibana et al. 2012). Among these leading candidates, *Pfs 25* and *Pvs25* have completed Phase I clinical trials with limited results (Wu et al. 2008). Transmission-blocking targets have been also identified in the *Anopheles* mosquito and include mosquito midgut ligands. The biggest advantage of targeting mosquito ligands is the possibility of obtaining a vaccine able to interrupt the transmission of more than one plasmodial species (Dinglasan and Jacobs-Lorena 2008).

## 5 Malaria Vector Control

Unlike other infections, and in particular from the other two major global public health threats HIV and TBC, malaria absolutely requires vectors to be transmitted. Vector control is indeed an essential and effective measure of malaria prevention and was responsible for malaria elimination from some countries in the past. Wide-scale use of insecticide-treated nets (ITNs) and indoor residual spraying (IRS) have contributed to the fall in malaria morbidity and mortality in the last decades (WHO 2013). ITNs should be used in all endemic areas; they possess several properties protecting the users (not only against mosquitoes but also against other insects carrying diseases) as well as the community by killing infective insects. IRS is effective for indoor night biters mosquitoes and not for outdoor daytime biters, thus its efficacy is mostly dependent on the insect's behavior.

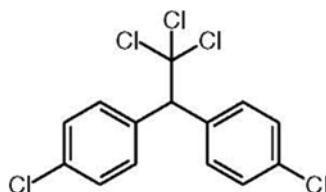
Pyrethroids are the only insecticides currently recommended by the WHO for use on bed nets (WHO 2013). It is therefore not surprising that during the last decades, pyrethroids-resistant mosquitoes have emerged in some areas of Africa (Ranson et al. 2011).

Three other classes of insecticides, organochlorines (OCs), organophosphates (OPs), carbamates (Cs), have been used in IRS throughout Africa, and resistance to all three had been reported in mosquitoes (WHO 2012).

### 5.1 Organochlorines

OCs belong to the class of chlorinated hydrocarbons, which are compounds that contain chlorine. The most famous compound of this class is Dichlorodiphenyltrichloroethane (DDT) (see Fig. 2.5), a pesticide with a long history of widespread use around the globe. OCs have the advantage to be cheap and very effective; however, they can have serious consequences on environment and on human health. OCs are indeed very stable compounds and can persist in the environment for long periods of time. Since they are lipophilic they can concentrate in body fat tissue and accumulate in animals and humans through the food chain (Kutz et al. 1991).

**Fig. 2.5** Chemical structure of DDT



DDT shares the same mechanism of action of pyrethroids, inhibiting the sodium channels. Insects with mutations in sodium channel gene can become resistant to DDT and to other related insecticides. DDT resistance is also conferred by overproduction of detoxification enzyme such as cytochrome P450 through enhanced biodegradation of the insecticide (Müller et al. 2008).

## 5.2 Organophosphates and Carbamates

OPs and Cs share the same mechanism of action, the inhibition of acetylcholinesterase, an enzyme responsible for neurotransmitter acetylcholine degradation at the cholinergic nerve synapse. Structural differences between the various OPs and Cs affect the efficiency and degree of acetylcholinesterase inhibition. With the spread of pyrethroid resistance, OPs and Cs may be vital alternative insecticides for IRS. However, resistance to OPs and Cs based on reduced sensitivity of acetylcholinesterase has been detected among *An. gambiae* from Cote d'Ivoire (N'Guessan et al. 2003).

## 5.3 Pyrethroids

Synthetic PYs represent one of the newer classes of insecticides and their use has increased significantly over the last 20 years. Although their chemical structure is different from that of OCs, their toxic effects are similar, acting on the insect's neuronal voltage-gated sodium ion channels in the axonal membranes of insect nerves. Resistance mechanisms are also shared by pyrethroids and DDT and cross-resistance between these compounds restricts the choice of alternative insecticides that can be used to manage resistance.

## 5.4 Transgenic Mosquitoes

Another approach for controlling transmission could be the use of genetically modified mosquitoes unable to transmit parasites. Several molecules able to block parasite transmission in mosquitoes have been investigated and competitor peptides able

to bind salivary gland receptors have been tested. *Anopheles stephensi*, genetically modified for the expression of the SM1 peptide able to bind an ookinete receptor on midgut epithelium, inhibited the ability of mosquito to transmit parasites (Ghosh et al. 2001). Other peptides displaying parasitocidal effects in mosquitoes such as cecropins, defensins, and scorpine have been also described (Gwadz et al. 1989; Kokoza et al. 2010). For a better explanation of these peptides see Chap. 7.

An interesting way to interfere with malaria transmission is the use of paratransgenesis for delivering anti-*Plasmodium* effector molecules by using genetically modified symbiotic microorganism of the insect (Hurwitz et al. 2011).

Lastly, the use of transgenic procedure can ameliorate the sterile insect technique (SIT) for anopheles mosquitoes. SIT approach relies on the release of radiation-sterilized males in the field to compete with wild ones. Currently, sterility in insects is induced by radiation; however, this approach can induce deleterious somatic effects leading to poor mating competitiveness of the sterile male compared to the wild insect (Benedict and Robinson 2003). The use of transgenic approach to induce sterility in male could increase the efficiency of SIT-based strategy (Catteruccia et al. 2009).

However, once a transgenic mosquito is obtained, the next step is its introduction in the field in order to substitute the wild vector population. In order to reach this focal point, several aspects, such as the potentially harmful ecological effects and the acceptance of genetically modified organism usage, have to be carefully investigated.

## 6 Conclusion

Despite many efforts, the burden of malaria is still great and to reach the goal of malaria elimination and eradication it is necessary to improve all the strategies to combat malaria. These include new diagnostic methods, drugs able to block the transmission of the disease, new classes of insecticides to control mosquitoes and an effective vaccine to prevent infection and/or transmission.

Progresses in each of these approaches could make a huge contribution to malaria eradication.

## References

- Agnandji ST, Lell B, Soulanoudjingar SS et al (2011) First results of phase 3 trial of RTS, S/AS01 malaria vaccine in African children. *N Engl J Med* 365:1863–1875
- Agnandji ST, Lell B, Fernandes JF et al (2012) A phase 3 trial of RTS, S/AS01 malaria vaccine in African infants. *N Engl J Med* 367:2284–2295
- Antinori S, Galimberti L, Milazzo L et al (2013) *Plasmodium knowlesi*: the emerging zoonotic malaria parasite. *Acta Trop* 125:191–201

- Barber BE, William T, Grigg MJ et al (2013) A prospective comparative study of knowlesi, falciparum, and vivax malaria in Sabah, Malaysia: high proportion with severe disease from Plasmodium knowlesi and Plasmodium vivax but no mortality with early referral and artesunate therapy. *Clin Infect Dis* 56:383–397
- Barker RH, Banchongaksorn T, Courval JM et al (1992) A simple method to detect Plasmodium falciparum directly from blood samples using the polymerase chain reaction. *Am J Trop Med Hyg* 46:416–426
- Benedict MQ, Robinson AS (2003) The first releases of transgenic mosquitoes: an argument for the sterile insect technique. *Trends Parasitol* 19:349–355
- Boström S, Ibitokou S, Oesterholt M et al (2012) Biomarkers of Plasmodium falciparum infection during pregnancy in women living in northeastern Tanzania. *PLoS One* 7:e48763
- Bray PG, Martin RE, Tilley L et al (2005) Defining the role of PfCRT in Plasmodium falciparum chloroquine resistance. *Mol Microbiol* 56:323–333
- Bronner U, Divis PC, Färnert A et al (2009) Swedish traveller with Plasmodium knowlesi malaria after visiting Malaysian Borneo. *Malar J* 8:15
- Brown PJ (1997) Malaria, miseria, and underpopulation in Sardinia: the “malaria blocks development” cultural model. *Med Anthropol* 17:239–254
- Bushby SR (1969) Combined antibacterial action in vitro of trimethoprim and sulphonamides. The in vitro nature of synergy. *Postgrad Med J* 45(suppl):10–18
- Catteruccia F, Crisanti A, Wimmer EA (2009) Transgenic technologies to induce sterility. *Malar J* 8(suppl 2):S7
- Clyde DF, Most H, McCarthy VC et al (1973) Immunization of man against sporozite-induced falciparum malaria. *Am J Med Sci* 266:169–177
- Coleman RE, Clavin AM, Milhous WK (1992) Gametocytocidal and sporontocidal activity of antimalarials against Plasmodium berghei ANKA in ICR mice and anopheles stephensi mosquitoes. *Am J Trop Med Hyg* 46:169–182
- Davis TM, Hung TY, Sim IK et al (2005) Piperaquine: a resurgent antimalarial drug. *Drugs* 65:75–87
- Dietze R, Perkins M, Boulous M et al (1995) The diagnosis of Plasmodium falciparum infection using a new antigen detection system. *Am J Trop Med Hyg* 52:45–49
- Dinglasan RR, Jacobs-Lorena M (2008) Flipping the paradigm on malaria transmission-blocking vaccines. *Trends Parasitol* 24:364–370
- Dondorp A, Nosten F, Stepniewska K et al (2005a) Artesunate versus quinine for treatment of severe falciparum malaria: a randomised trial. *Lancet* 366:717–725
- Dondorp AM, Desakorn V, Pongtavornpinyo W et al (2005b) Estimation of the total parasite biomass in acute falciparum malaria from plasma PfHRP2. *PLoS Med* 2:e204
- Dondorp AM, Fanello CI, Hendriksen IC et al (2010) Artesunate versus quinine in the treatment of severe falciparum malaria in African children (AQUAMAT): an open-label, randomised trial. *Lancet* 376:1647–1657
- Eckstein-Ludwig U, Webb RJ, Van Goethem ID et al (2003) Artemisinins target the SERCA of Plasmodium falciparum. *Nature* 424:957–961
- Egan TJ (2008) Haemozoin formation. *Mol Biochem Parasitol* 157:127–136
- Epstein JE, Tewari K, Lyke KE et al (2011) Live attenuated malaria vaccine designed to protect through hepatic CD8<sup>+</sup> T cell immunity. *Science* 334:475–480
- Fitch CD, Cai GZ, Chen YF et al (2003) Relationship of chloroquine-induced redistribution of a neutral aminopeptidase to hemoglobin accumulation in malaria parasites. *Arch Biochem Biophys* 410:296–306
- Fowkes FJ, Richards JS, Simpson JA et al (2010) The relationship between anti-merozoite antibodies and incidence of Plasmodium falciparum malaria: a systematic review and meta-analysis. *PLoS Med* 7:e1000218
- Gamboa D, Ho MF, Bendezu J et al (2010) A large proportion of P. falciparum isolates in the Amazon region of Peru lack pfhrp2 and pfhrp3: implications for malaria rapid diagnostic tests. *PLoS One* 5:e8091

- Ghosh AK, Ribolla PE, Jacobs-Lorena M (2001) Targeting Plasmodium ligands on mosquito salivary glands and midgut with a phage display peptide library. *Proc Natl Acad Sci U S A* 98:13278–13281
- Goodman AL, Draper SJ (2010) Blood-stage malaria vaccines—recent progress and future challenges. *Ann Trop Med Parasitol* 104:189–211
- Gordon DM, McGovern TW, Krzych U et al (1995) Safety, immunogenicity, and efficacy of a recombinantly produced Plasmodium falciparum circumsporozoite protein-hepatitis B surface antigen subunit vaccine. *J Infect Dis* 171:1576–1585
- Greenwood B (2005) Malaria vaccines. Evaluation and implementation. *Acta Trop* 95:298–304
- Greenwood BM, Fidock DA, Kyle DE et al (2008) Malaria: progress, perils, and prospects for eradication. *J Clin Invest* 118:1266–1276
- Grobusch MP, Egan A, Gosling RD et al (2007) Intermittent preventive therapy for malaria: progress and future directions. *Curr Opin Infect Dis* 20:613–620
- Gwadz RW, Kaslow D, Lee JY et al (1989) Effects of magainins and cecropins on the sporogonic development of malaria parasites in mosquitoes. *Infect Immun* 57:2628–2633
- Han ET, Watanabe R, Sattabongkot J et al (2007) Detection of four Plasmodium species by genus- and species-specific loop-mediated isothermal amplification for clinical diagnosis. *J Clin Microbiol* 45:2521–2528
- Haynes RK (2006) From artemisinin to new artemisinin antimalarials: biosynthesis, extraction, old and new derivatives, stereochemistry and medicinal chemistry requirements. *Curr Top Med Chem* 6:509–537
- Haynes RK, Chan WC, Wong HN et al (2010) Facile oxidation of leucomethylene blue and dihydroflavins by artemisinins: relationship with flavoenzyme function and antimalarial mechanism of action. *ChemMedChem* 5:1282–1299
- Haynes RK, Cheu KW, Chan HW et al (2012) Interactions between artemisinins and other antimalarial drugs in relation to the cofactor model—a unifying proposal for drug action. *ChemMedChem* 7:2204–2226
- Higgins SJ, Xing K, Kim H et al (2013) Systemic release of high mobility group box 1 (HMGB1) protein is associated with severe and fatal Plasmodium falciparum malaria. *Malar J* 12:105
- Hoppe HC, Van Schalkwyk DA, Wiehart UIM et al (2004) Antimalarial quinolines and artemisinin inhibit endocytosis in Plasmodium falciparum. *Antimicrob Agents Chemother* 48:2370–2378
- Houzé S, Hubert V, Le Pessec G et al (2011) Combined deletions of pfhrp2 and pfhrp3 genes result in Plasmodium falciparum malaria false-negative rapid diagnostic test. *J Clin Microbiol* 49:2694–2696
- Hurwitz I, Fieck A, Read A et al (2011) Paratransgenic control of vector borne diseases. *Int J Biol Sci* 7:1334–1344
- Hviid L (2010) The role of Plasmodium falciparum variant surface antigens in protective immunity and vaccine development. *Hum Vaccin* 6:84–89
- Iqbal J, Khalid N, Hira PR (2002) Comparison of two commercial assays with expert microscopy for confirmation of symptomatically diagnosed malaria. *J Clin Microbiol* 40:4675–4678
- Iseki H, Kawai S, Takahashi N et al (2010) Evaluation of a loop-mediated isothermal amplification method as a tool for diagnosis of infection by the zoonotic simian malaria parasite Plasmodium knowlesi. *J Clin Microbiol* 48:2509–2514
- Jani D, Nagarkatti R, Beatty W et al (2008) HDP-a novel heme detoxification protein from the malaria parasite. *PLoS Pathog* 4:e1000053
- Jepsen MP, Jogdand PS, Singh SK et al (2013) The malaria vaccine candidate GMZ2 elicits functional antibodies in individuals from malaria endemic and non-endemic areas. *J Infect Dis* 208:479–488
- Kattenberg JH, Versteeg I, Migchelsen SJ et al (2012) New developments in malaria diagnostics: monoclonal antibodies against Plasmodium dihydrofolate reductase-thymidylate synthase, heme detoxification protein and glutamate rich protein. *MAbs* 4:120–126
- Khadjavi AG, Prato M (2010) From control to eradication of malaria: the end of being stuck in second gear? *Asian Pac J Trop Med* 3:412–420

- Kilian AH, Metzger WG, Mutschelknauss EJ et al (2000) Reliability of malaria microscopy in epidemiological studies: results of quality control. *Trop Med Int Health* 5:3–8
- Kokoza V, Ahmed A, Woon Shin S et al (2010) Blocking of Plasmodium transmission by cooperative action of Cecropin A and Defensin A in transgenic Aedes aegypti mosquitoes. *Proc Natl Acad Sci U S A* 107:8111–8116
- Krungkrai J, Burat D, Kudan S et al (1999) Mitochondrial oxygen consumption in asexual and sexual blood stages of the human malarial parasite, Plasmodium falciparum. *Southeast Asian J Trop Med Public Health* 30:636–642
- Kumar N, Pande V, Bhatt RM et al (2013) Genetic deletion of HRP2 and HRP3 in Indian Plasmodium falciparum population and false negative malaria rapid diagnostic test. *Acta Trop* 125:119–121
- Kutz FW, Wood PH, Bottimore DP (1991) Organochlorine pesticides and polychlorinated biphenyls in human adipose tissue. *Rev Environ Contam Toxicol* 120:1–82
- Lau YL, Fong MY, Mahmud R et al (2011) Specific, sensitive and rapid detection of human plasmodium knowlesi infection by loop-mediated isothermal amplification (LAMP) in blood samples. *Malar J* 10:197
- Legrand E, Volney B, Meynard JB et al (2008) In vitro monitoring of Plasmodium falciparum drug resistance in French Guiana: a synopsis of continuous assessment from 1994 to 2005. *Antimicrob Agents Chemother* 52:288–298
- Longo M, Zanoncelli S, Torre PD et al (2006) In vivo and in vitro investigations of the effects of the antimalarial drug dihydroartemisinin (DHA) on rat embryos. *Reprod Toxicol* 22:797–810
- Mayxay M, Pukrittayakamee S, Chotivanich K et al (2001) Persistence of Plasmodium falciparum HRP-2 in successfully treated acute falciparum malaria. *Trans R Soc Trop Med Hyg* 95:179–182
- Mayxay M, Pukrittayakamee S, Newton PN et al (2004) Mixed-species malaria infections in humans. *Trends Parasitol* 20:233–240
- Mayxay M, Barends M, Brockman A et al (2007) In vitro antimalarial drug susceptibility and pfprt mutation among fresh Plasmodium falciparum isolates from the Lao PDR (Laos). *Am J Trop Med Hyg* 76:245–250
- Meshnick SR, Thomas A, Ranz A et al (1991) Artemisinin (qinghaosu): the role of intracellular hemin in its mechanism of antimalarial action. *Mol Biochem Parasitol* 49:181–189
- Mharakurwa S, Shiff CJ (1997) Post treatment sensitivity studies with the ParaSight-F test for malaria diagnosis in Zimbabwe. *Acta Trop* 66:61–67
- Minota S, Cameron B, Welch WJ et al (1988) Autoantibodies to the constitutive 73-kD member of the hsp70 family of heat shock proteins in systemic lupus erythematosus. *J Exp Med* 168:1475–1480
- Mouatcho JC, Goldring JP (2013) Malaria rapid diagnostic tests: challenges and prospects. *J Med Microbiol* 62:1491–1505
- Mueller I, Betuela I, Ginny M et al (2007) The sensitivity of the OptiMAL rapid diagnostic test to the presence of Plasmodium falciparum gametocytes compromises its ability to monitor treatment outcomes in an area of Papua New Guinea in which malaria is endemic. *J Clin Microbiol* 45:627–630
- Müller P, Warr E, Stevenson BJ et al (2008) Field-caught permethrin-resistant Anopheles gambiae overexpress CYP6P3, a P450 that metabolises pyrethroids. *PLoS Genet* 4:e1000286
- Mutanda LN (1999) Assessment of drug resistance to the malaria parasite in residents of Kampala, Uganda. *East Afr Med J* 76:421–424
- N'Guessan R, Darriet F, Guillet P et al (2003) Resistance to carbosulfan in Anopheles gambiae from Ivory Coast, based on reduced sensitivity of acetylcholinesterase. *Med Vet Entomol* 17:19–25
- Na BK, Park JW, Lee HW et al (2007) Characterization of Plasmodium vivax heat shock protein 70 and evaluation of its value for serodiagnosis of tertian malaria. *Clin Vaccine Immunol* 14:320–322
- Nardin EH, Nussenzweig RS (1993) T cell responses to pre-erythrocytic stages of malaria: role in protection and vaccine development against pre-erythrocytic stages. *Annu Rev Immunol* 11:687–727

- Nguyen PH, Day N, Pram TD et al (1995) Intraleucocytic malaria pigment and prognosis in severe malaria. *Trans R Soc Trop Med Hyg* 89:200–204
- Nussenzweig RS, Vanderberg J, Most H et al (1967) Protective immunity produced by the injection of x-irradiated sporozoites of *Plasmodium berghei*. *Nature* 216:160–162
- O'Neill PM, Bray PG, Hawley SR et al (1998) 4-Aminoquinolines—past, present, and future: a chemical perspective. *Pharmacol Ther* 77:29–58
- O'Neill PM, Ward SA, Berry NG et al (2006) A medicinal chemistry perspective on 4-aminoquinoline antimalarial drugs. *Curr Top Med Chem* 6:479–507
- O'Neill PM, Barton VE, Ward SA (2010) The molecular mechanism of action of artemisinin—the debate continues. *Molecules* 15:1705–1721
- Oddoux O, Debourgogne A, Kantele A et al (2011) Identification of the five human *Plasmodium* species including *P. knowlesi* by real-time polymerase chain reaction. *Eur J Clin Microbiol Infect Dis* 30:597–601
- Olliaro P (2001) Mode of action and mechanisms of resistance for antimalarial drugs. *Pharmacol Ther* 89:207–219
- Olliaro PL, Haynes RK, Meunier B et al (2001) Possible modes of action of the artemisinin-type compounds. *Trends Parasitol* 17:122–126
- Palmer CJ, Lindo JF, Klaskala WI et al (1998) Evaluation of the OptiMAL test for rapid diagnosis of *Plasmodium vivax* and *Plasmodium falciparum* malaria. *J Clin Microbiol* 36:203–206
- Partnership RBM (2008) The Global Malaria Action Plan—for a malaria free world.
- Peters W (1982) Antimalarial drug resistance: an increasing problem. *Br Med Bull* 38:187–192
- Poon LL, Wong BW, Ma EH et al (2006) Sensitive and inexpensive molecular test for *falciparum* malaria: detecting *Plasmodium falciparum* DNA directly from heat-treated blood by loop-mediated isothermal amplification. *Clin Chem* 52:303–306
- Posner GH, Oh CH, Gerena L et al (1992) Extraordinarily potent antimalarial compounds: new, structurally simple, easily synthesized, tricyclic 1,2,4-trioxanes. *J Med Chem* 35:2459–2467
- Pradel G (2007) Proteins of the malaria parasite sexual stages: expression, function and potential for transmission blocking strategies. *Parasitology* 134:1911–1929
- Pradines B, Mabika Mamfoumbi M, Parzy D et al (1998) In vitro susceptibility of Gabonese wild isolates of *Plasmodium falciparum* to artemether, and comparison with chloroquine, quinine, halofantrine and amodiaquine. *Parasitology* 117(Pt 6):541–545
- Price RN, Douglas NM, Anstey NM (2009) New developments in *Plasmodium vivax* malaria: severe disease and the rise of chloroquine resistance. *Curr Opin Infect Dis* 22:430–435
- Ranson H, N'Guessan R, Lines J et al (2011) Pyrethroid resistance in African anopheline mosquitoes: what are the implications for malaria control? *Trends Parasitol* 27:91–98
- Raynes K (1999) Bisquinoline antimalarials: their role in malaria chemotherapy. *Int J Parasitol* 29:367–379
- Richards JS, Beeson JG (2009) The future for blood-stage vaccines against malaria. *Immunol Cell Biol* 87:377–390
- Ridley RG, Dorn A, Vippagunta SR et al (1997) Haematin (haem) polymerization and its inhibition by quinoline antimalarials. *Ann Trop Med Parasitol* 91:559–566
- Rieckmann KH, Carson PE, Beaudoin RL et al (1974) Letter: sporozoite induced immunity in man against an Ethiopian strain of *Plasmodium falciparum*. *Trans R Soc Trop Med Hyg* 68:258–259
- Roberts L, Egan TJ, Joiner KA et al (2008) Differential effects of quinoline antimalarials on endocytosis in *Plasmodium falciparum*. *Antimicrob Agents Chemother* 52:1840–1842
- Rogerson SJ, Hviid L, Duffy PE et al (2007) Malaria in pregnancy: pathogenesis and immunity. *Lancet Infect Dis* 7:105–117
- Sattabongkot J, Tsuboi T, Hisaeda H et al (2003) Blocking of transmission to mosquitoes by antibody to *Plasmodium vivax* malaria vaccine candidates Pvs25 and Pvs28 despite antigenic polymorphism in field isolates. *Am J Trop Med Hyg* 69:536–541
- Seder RA, Chang LJ, Enama ME et al (2013) Protection against malaria by intravenous immunization with a nonreplicating sporozoite vaccine. *Science* 341:1359–1365



- Sharma YD (1992) Structure and possible function of heat-shock proteins in *Falciparum malaria*. *Comp Biochem Physiol B* 102:437–444
- Sherman IW (1979) Biochemistry of *Plasmodium* (malarial parasites). *Microbiol Rev* 43:453–495
- Sinden RE (2010) A biologist's perspective on malaria vaccine development. *Hum Vaccin* 6:3–11
- Snounou G, Viriyakosol S, Jarra W et al (1993) Identification of the four human malaria parasite species in field samples by the polymerase chain reaction and detection of a high prevalence of mixed infections. *Mol Biochem Parasitol* 58:283–292
- Srivastava IK, Morrissey JM, Darrouzet E et al (1999) Resistance mutations reveal the atovaquone-binding domain of cytochrome b in malaria parasites. *Mol Microbiol* 33:704–711
- Sutherland CJ (2009) Surface antigens of *Plasmodium falciparum* gametocytes—a new class of transmission-blocking vaccine targets? *Mol Biochem Parasitol* 166:93–98
- Tachibana M, Sato C, Otsuki H et al (2012) *Plasmodium vivax* gametocyte protein Pvs230 is a transmission-blocking vaccine candidate. *Vaccine* 30:1807–1812
- Thévenon AD, Zhou JA, Megnekou R et al (2010) Elevated levels of soluble TNF receptors 1 and 2 correlate with *Plasmodium falciparum* parasitemia in pregnant women: potential markers for malaria-associated inflammation. *J Immunol* 185:7115–7122
- Tinto H, Rwagacondo C, Karema C et al (2006) In-vitro susceptibility of *Plasmodium falciparum* to monodesethylamodiaquine, dihydroartemisinin and quinine in an area of high chloroquine resistance in Rwanda. *Trans R Soc Trop Med Hyg* 100:509–514
- Touré AO, Koné LP, Jambou R et al (2008) In vitro susceptibility of *P. falciparum* isolates from Abidjan (Côte d'Ivoire) to quinine, artesunate and chloroquine. *Sante* 18:43–47
- Vaidya AB, Lashgari MS, Pologe LG et al (1993) Structural features of *Plasmodium* cytochrome b that may underlie susceptibility to 8-aminoquinolines and hydroxynaphthoquinones. *Mol Biochem Parasitol* 58:33–42
- Vanbuskirk KM, O'Neill MT, De La Vega P et al (2009) Preerythrocytic, live-attenuated *Plasmodium falciparum* vaccine candidates by design. *Proc Natl Acad Sci U S A* 106:13004–13009
- Vennerstrom JL, Ellis WY, Ager AL et al (1992) Bisquinolines. 1. N, N-bis(7-chloroquinolin-4-yl) alkanediamines with potential against chloroquine-resistant malaria. *J Med Chem* 35:2129–2134
- Vennerstrom JL, Nuzum EO, Miller RE et al (1999) 8-Aminoquinolines active against blood stage *Plasmodium falciparum* in vitro inhibit hemozoin polymerization. *Antimicrob Agents Chemother* 43:598–602
- Vinayak S, Rathore D, Kariuki S et al (2009) Limited genetic variation in the *Plasmodium falciparum* heme detoxification protein (HDP). *Infect Genet Evol* 9:286–289
- Warhurst DC, Williams JE (1996) ACP Broadsheet no 148. July 1996. Laboratory diagnosis of malaria. *J Clin Pathol* 49:533–538
- Wellems TE, Plowe CV (2001) Chloroquine-resistant malaria. *J Infect Dis* 184:770–776
- White NJ, Pukrittayakamee S, Hien TT et al (2014) Malaria. *Lancet* 383:723–735
- WHO (2010) Guidelines for the treatment of malaria. WHO, Geneva
- WHO (2011) Malaria rapid diagnostic test performance: summary results of WHO malaria RDT product testing: rounds 1–3 (2008–2011)
- WHO (2012) Global plan for insecticide resistance management in malaria vectors. WHO, Geneva
- WHO (2013) World malaria report. WHO, Geneva
- Wilson ML (2012) Malaria rapid diagnostic tests. *Clin Infect Dis* 54:1637–1641
- Wu Y, Ellis RD, Shaffer D et al (2008) Phase 1 trial of malaria transmission blocking vaccine candidates Pfs25 and Pvs25 formulated with montanide ISA 51. *PLoS One* 3:e2636
- Yuvaniyama J, Chitnumsub P, Kamchonwongpaisan S et al (2003) Insights into antifolate resistance from malarial DHFR-TS structures. *Nat Struct Biol* 10:357–365
- Zhang M, Hisaeda H, Kano S et al (2001) Antibodies specific for heat shock proteins in human and murine malaria. *Microbes Infect* 3:363–367

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