Malaria

Authors

Margaret A. Phillips[1], Jeremy N. Burrows[2], Christine Manyando[3], Rob Hooft van Huijsduijnen[2] Wesley C. Van Voorhis[4] and Timothy N. C. Wells[2]

[1]Departments of Biochemistry, University of Texas Southwestern Medical Center at Dallas, 5323 Harry Hines Blvd, Dallas, Texas 75390-9038, U.S.A; [2]Medicines for Malaria Venture, Geneva, Switzerland; [3]Tropical Diseases Research Centre, Ndola, Zambia, [4]University of Washington, Department of Medicine, Division of Allergy and Infectious Diseases, Center for Emerging and Re-emerging Infectious Diseases, Seattle, Washington, U.S.A.

Correspondence to:

M.A.P.

margaret.phillips@UTSouthwestern.edu.

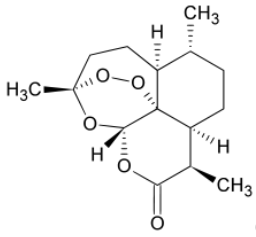
Abstract

Malaria is caused in humans by five species of single-cell, eukaryotic *Plasmodium* parasites (mainly *Plasmodium falciparum* and *Plasmodium vivax*) that are transmitted by the bite of *Anopheles* mosquitoes. Malaria remains one of the most serious infectious diseases, globally threatening nearly half of the world population and leading to an estimated half a million deaths in 2015, predominantly among children in Africa. Malaria is managed through a combination of vector control approaches (such as insecticide spraying and the use of insecticide-treated bed nets) and drugs for both treatment and prevention. Wide-spread use of artemisinin-based combination therapies has contributed to substantial declines in malaria-related deaths; however, the emergence of drug resistance threatens to reverse this progress. Advances in the understanding of the underlying molecular basis of pathogenesis have fuelled the development of new diagnostics, drugs and insecticides. Several new combination therapies are in clinical development that have efficacy against drug-resistant parasites and the potential to be used in single dose regimens to improve compliance. This ambitious programme to eliminate malaria also includes new approaches that could yield malaria vaccines or novel vector control strategies. However, despite these achievements, a well-coordinated, global effort on multiple fronts is needed if malaria elimination is to be achieved.

[H1] Introduction

Malaria has had a profound effect on human lives for thousands of years and remains one of the most one of the most serious, life-threatening infectious diseases [1-3]. The disease is caused by protozoan protozoan pathogens of the *Plasmodium* species; *Plasmodium falciparum* (*P. falciparum*) and *Plasmodium vivax* (*P. vivax*), for which humans are the exclusive mammalian hosts, are the most most common and are responsible for the largest public health burden. Malaria is transmitted by the bite the bite of *Plasmodium*-infected female mosquitoes of the *Anopheles* genus[1-3]. During a blood meal, meal, infected mosquitoes inject, along with their anticoagulating saliva, sporozoites, which are the the infective, motile spore-like stage of *Plasmodium*. Sporozoites journey through the skin to the vasculature and into hepatocytes in the liver (Figure 1

Youyou Tu was recognized by the 2015 Nobel Prize committee for her contribution to medicine for the discovery of artemisinin, by retrieving and following instructions from ancient Chinese texts [250]. Thanks to the ability of artemisinin to rapidly reduce parasitemia and fever, the effect that artemisinin and its derivatives had on the management of malaria cannot be overstated: since their introduction in the 1970s and subsequent wider implementation, which was possible particularly owing to the work of Prof. Nicholas White and colleagues [251-254], millions of lives were saved. These drugs appear to be activated by heme derived iron and their toxicity is probably mediated through the formation of reactive oxidative radicals[43]. Data suggest that they interfere with phosphatidylinositol-3-phosphate (PI3P) metabolism (which is thought to be involved in the trafficking of haemoglobin to the digestive vacuole[255]) and provide possible mechanistic insight into the nature of clinically observed artemisinin resistance[256].



Chemical structure of artemisinin

Figure 1). In the hepatocyte, a single sporozoite can generate tens of thousands of merozoites (the stage that results from multiple asexual fissions (schizogony) of a sporozoite within the body of the host), which are released from the hepatocytes into the blood stream where they enter red blood cells to replicate (erythrocytic schizogony). A fraction of merozoites (sexually committed) also differentiate and mature into male and female gametocytes, which is the stage that infects the mosquito host when it takes a blood meal [4,5]. The onset of clinical symptoms generally occurs 7-10 days after the initial mosquito bite. *P. vivax and Plasmodium ovale (P. ovale)* also have dormant forms, called hypnozoites, which can emerge from the liver years after the initial inoculation[6], leading to relapse if not treated properly.

The consequences of *Plasmodium* infection vary in severity depending on the species and on host factors, including the level of host immunity, which is linked to the past extent of parasite exposure [7,8]. Malaria is usually classified as asymptomatic, uncomplicated or severe (complicated) [9]. (Box 1) Typical initial symptoms are low-grade fever, shaking chills, muscle aches and in children digestive symptoms. These symptoms can present suddenly (paroxysms), and then progress to drenching sweats, high fever and exhaustion. Malaria paroxysmal symptoms are manifest after haemolysis of *Plasmodium*-invaded red blood cells. Severe malaria is often fatal and presents with severe anaemia, and various manifestations of multi-organ damage, which can include cerebral malaria[8] (Box 1). Severe malaria complications are due to microvascular obstruction caused by the presence of red blood cell stage parasites in capillaries[8,10,11]. This review will focus on our understanding of malaria pathology in the context of parasite and vector biology, progress in diagnostics and new treatments (drugs and vaccines), chemoprotection and chemoprevention.

[H1] Epidemiology

[H2] Vector

Human malaria parasites are transmitted exclusively by about 40 species of the mosquito genus *Anopheles* [12]. During *Anopheles* mating, males transfer high levels of the steroid hormone 20-hydroxyecdysone to the female, and the presence of this hormone has been associated with favourable conditions for *Plasmodium* development [13]. Malaria-competent *Anopheles* species are abundant and distributed all over the globe, including the Arctic. However, the efficacy of malaria transmission depends on the vector species and, therefore, varies considerably worldwide; for example, in tropical Africa *A. gambiae* is a major and highly efficient vector[14]. The first WHO Global Malaria Eradication Programme (1955-1972) involved, besides chloroquine-based treatments, large-scale insecticide campaigns using dichlorodiphenyltrichloroethane (DDT)[15]. This strategy was quite effective against *P. falciparum*; although the mosquitoes gradually repopulated DDT-treated areas (because they developed resistance to the insecticide, and the use of DDT itself waned owing to its costs and increasing environmental concerns), these areas have often remained malaria-free, sometimes until present. More-selective vector-control approaches, such as the use of insecticide-treated bed nets and indoor residual spraying, have eliminated malaria from several areas (see Prevention). However, mosquito resistance to insecticides is a growing concern. Of the 78 countries that monitor mosquito resistance to insecticides, 60 have reported resistance to one or more insecticides since 2010(Ref. [16]).

[H2] Parasite

Plasmodium species are single-celled eukaryotic organisms [17-19] that belong to the phylum Apicomplexa, which is named for the apical complex that is involved in host cell invasion. A discussion of the parasite genome and the genetic approaches used to study parasite biology is provided in Box 2. Of the five human infective *Plasmodium* species, *P. falciparum* causes the bulk of malaria-associated

morbidity and mortality in sub-Saharan Africa, which peaked in the late nineties at over a million deaths annually in the continent[20] (Figure 2). *P. falciparum* is associated with severe malaria and complications in pregnancy (Box 3); most malaria-related deaths are associated with this species, which kills about 1,200 African children aged under five each day[21]. However, *P. falciparum* is also found in malarious tropical areas around the world. *P. vivax* is found in malarious areas around the world, and generally accounts for the majority of malaria cases in Central and South America and in temperate climates. This distribution can be explained by the fact that *P. vivax* can travel across climatically unfavourable regions and can stay dormant in hypnozoite form in its human host's liver for many years. Furthermore, many Africans are negative for the Duffy antigen on the surface of red blood cells, and this genotype provides protection from *P. vivax* malaria, making the fixation and the penetration of *P. vivax* in the red blood cell more difficult.[22] However, some cases of *P. vivax* transmission to Duffy negative individuals have been reported suggesting alternative mechanisms of invasion might be present in some strains and this might portend the escalation of *P. vivax* malaria to Africa.[23,24] *P. ovale* is also found in Africa and Asia, but is especially prevalent in West Africa. Two sympatric species exist, *P.o. curtisi* and *P.o. wallikeri*[25]. *Plasmodium malariae* (*P. malariae)*, which can be found worldwide but is especially prevalent in West Africa, causes the mildest infections, although it has been associated with splenomegaly or renal damage upon chronic infection. *Plasmodium knowlesi*, initially considered a parasite of non-human primates, can not only cause malaria in humans, but also lead to severe and even fatal malaria complications[26,27]. The reasons for the emergence of *Plasmodium knowlesi* in humans are not yet fully understood but are possibly linked to land use changes that have brought humans in close contact with *P. knowlesi* infected mosquitos [28]. Regardless the possible emergence of a form of malaria as a zoonosis poses obvious complications for elimination. Additionally, coinfections between *P. falciparum* and *P. vivax* have been well documented and have been reported to occur in up to 10-30% of patients living in areas where both parasites are prevalent [29,30]. Mixed infections can also include other

133 species such as *P. ovale* and *P. malariae*, and newer diagnostic methods are being developed
134 that will allow better assessment of the frequency and distribution of these types of coinfections
135 (e.g. [31]).

136 **[H2] Disease**

137 Malaria remains a major burden to people residing in resource-limited areas in Africa,
138 Asia and Central and South America (Figure 2). An estimated 214 million cases of malaria
139 occurred in 2015 (Ref. [16]). Africa bears the brunt of the burden, with 88% of the cases, followed
140 by South East Asia (10%), the eastern Mediterranean region (2%), and Central and South
141 America (<1%). Malaria continues to kill over three times as many people as all armed conflicts;
142 in 2015, there were an estimated 438,000 (Ref. [16]) – 631,000 (Ref. [32]) deaths resulting from
143 malaria, compared with an estimated 167,000 deaths due to armed conflicts [33,34]. In areas of
144 continuous transmission of malaria, children <5 years and the foetuses of infected pregnant
145 women experience the most morbidity and mortality from the disease. Children older than six
146 months are particularly susceptible because they have lost their maternal antibodies but have
147 not yet developed protective immunity. In fact, adults and children over 5 years of age who live
148 in regions of year round *P. falciparum* transmission develop a partial protective immunity due to
149 repeated exposure to the parasite. There is evidence that immunity against *P. vivax* is acquired
150 more quickly[35]. Individuals with low protective immunity against *P. falciparum* are particularly
151 vulnerable to severe malaria. Severe malaria occurs in only 1% of infections in African children
152 and is more-common in patients who lack strong immune protection (for example, individuals
153 who live in low-transmission settings, children <5 years of age and naïve hosts). Severe malaria
154 is deadly in 10% of children and 20% of adults[7]. Pregnant women are more susceptible to
155 *Plasmodium* infection because the placenta itself selects for the emergence of parasites that
156 express receptors that recognize the placental vasculature; these receptors are antigens to
157 which pregnant women have not yet become partially immune [7] (Box 3). This vulnerability

158 increases the risk of miscarriage, and parasitemia in the placenta can have adverse effects on
159 the foetus [36-38] (Box 3).

160 Co-infection of *Plasmodium* with other pathogens is common, including HIV,
161 *Mycobacterium tuberculosis* and helminths. HIV-infected adults are at increased risk of severe
162 malaria and death[39]. The overall prevalence of helminth infection is very high (>50% of the
163 population) in malaria-endemic regions and was associated with increased malaria
164 parasitaemia[40]. Surprisingly, naturally occurring iron deficiency and anaemia protect from
165 severe malaria, an unexpected finding[41], since numerous clinical studies aimed at fortifying
166 children and preventing anaemia by distributing iron supplements[42].

167 From 2000 to 2015, the incidence of malaria fell by 37% and malaria deaths by 60%
168 globally[16]. The WHO attributes much of this reduction of malaria-associated morbidity and
169 mortality to the scale-up of three interventions: insecticide-treated bed nets (69% of the
170 reduction), artemisinin-based combination therapies (ACTs; 21%) and indoor-residual
171 insecticide spraying (10%)[16] (see Prevention). Until ACT was introduced, progress on malaria
172 control in most malarious countries was threatened or reversed by the nearly world-wide
173 emergence of chloroquine-resistant and sulfadoxine-pyrimethamine -resistant *P. falciparum*
174 strains, and more recently, of other resistant *Plasmodium* species. ACT has become the
175 antimalarial medicine of choice in most malarious areas, demonstrating rapid parasite
176 clearance, superior efficacy (compared with other clinically approved drugs), and >98% cure
177 rates (typically defined as the percentage of patients who remain malaria-free for 28 days; re-
178 infection events do not count as a recurrence). ACTs achieve these results even in strains
179 resistant to older antimalarials — effectively turning the tide against antimalarial drug-resistance.
180 However, the emergence of artemisinin-resistant strains in South East Asia threatens the
181 usefulness of ACTs [43-46] (see Drug resistance).

182

[H1] Mechanisms/pathophysiology

[H2] Red blood cell stage

As previously mentioned, the red blood cell stage of *Plasmodium* infection is the cause of symptomatic malaria, as red blood cells are the site of abundant parasite replication.

[H3] Invasion. *Plasmodium* parasites gain entrance to the red blood cell through specific ligand-receptor interactions mediated by proteins on the surface of the parasite that interact with receptors on the host erythrocyte (mature red blood cell) or reticulocyte (immature red blood cell) (Figure 3) [47]. Whereas *P. falciparum* can invade and replicate in erythrocytes and reticulocytes, *P. vivax* and other species predominantly invade reticulocytes, which are less abundant than erythrocytes[48]. Most of the parasite erythrocyte or reticulocyte binding proteins that have been associated with invasion are redundant or are expressed as a family of variant forms; however, for *P. falciparum* two essential red blood cell receptors (basigin and complement decay-accelerating factor (CD55)) have been identified (Figure 3).

[H3] Replication. Once *Plasmodium* gains entry into the red blood cell, it exports hundreds of proteins into the host cell cytoplasm and cell surface that modulate the acquisition of nutrients, cell adhesion and sequestration in tissues and pathogenesis.[3,49,50] Molecular and cell biology approaches are expanding our understanding of the molecular machinery required for the export, identify and function of these proteins.

In the red blood cell, *Plasmodium* replicates rapidly, and during symptomatic disease parasites typically grow exponentially up to around 10^{11}-10^{12} per patient. This rapid growth requires sustained pools of nucleotides for the synthesis of DNA and RNA and, as a consequence, numerous anti-malarials target pyrimidine biosynthesis[51]. (Figure 3) *Plasmodium* is auxotrophic for all of the amino acids it needs (*i.e.* it must acquire all of these from its food because it cannot synthesize them from other precursors). Haemoglobin digestion (in a

specialized food vacuole) supplies all amino acids except isoleucine, which must be obtained from other host cell components [52]. Haemoglobin digestion also releases heme, which is toxic to the parasite and, therefore, is polymerized into hemozoin (often called malaria pigment, which is visible as blue pigment under light microscopy), an insoluble crystal that sequesters the toxic metabolite[53]. How heme polymerization is facilitated by the parasite remains unclear. A complex of several proteases and heme detoxification protein (HDP) have been identified in the food vacuole; follow up studies *in vitro* showed that components of this complex (for example, falcipain 2, HDP and lipids) were able to catalyse the conversion[54]. The importance of understanding this mechanism is highlighted by the finding that chloroquine and other antimalarials act by inhibiting heme polymerization [55](Figure 3). There is also evidence that the iron (heme-bound or free) liberated in the food vacuole during haemoglobin digestion plays a part in activating the toxicity to the parasite of artemisinins [43].

Nutrient uptake by the parasite is coupled to the detrimental accumulation of sodium (Na^+); however, the parasite expresses an essential plasma membrane Na^+ export pump (the cation ATPase *Pf*ATP4) that can maintain Na^+ homeostasis (Figure 3). [56-58]. Remodelling of the plasma membrane (membrane ingression) to generate daughter merozoites in the late schizont stage requires phosphatidylinositol-4 kinase (PfPI(4)K) [59]. Both PfPI(4)K and PfATP4 are targets of new drugs under development (Figure 3).

[H2] Immune evasion and host immunity

Malaria parasites first encounter the host immune system when sporozoites are injected in the skin (measure to be ~15 per mosquito bite in one study[60]), where they are phagocytosed by dendritic cells that then transport them to the lymph node draining the skin inoculation site[61]. The chances of transmission are increased when the host is bitten by mosquitoes that carry a

larger number of sporozoites, despite the fact that the number of sporozoites that can simultaneously pass through the proximal duct is limited by the duct diameter [62]. Sporozoites encounter a number of effectors of the immune system and how a minority of them can reach the liver and infect the hepatocytes is not well understood. Immune evasion in the liver could be in part explained by the ability of sporozoites to suppress the function of Kupffer cells (or stellate macrophages, the liver's resident macrophages) and repress the expression of MHC Class I genes [63]. Our understanding of host immunity associated with the red blood cell stage is more complete. Virulence genes in *Plasmodium* species are part of large expanded multigene families that are found in specialized (for example, sub-telomeric) regions of the chromosomes.[7,64,65] These gene families (for example, *var* genes in *P. falciparum*) encode variants of cell surface proteins that function in immune evasion through antigenic variation and also are involved in mediating cytoadherence of infected red blood cells to endothelial cells leading to sequestration in tissues.

Malaria disease severity both in terms of parasite burden and the risk for complicated malaria are dependent on the levels of protective immunity acquired by the human host[66-68], which can help to decrease the severity of symptoms and reduce the risk of severe malaria. Immunity is thought to result from circulating IgG antibodies against surface proteins on sporozoites (thereby blocking hepatocyte invasion) and merozoites (blocking red blood cell invasion). In high-transmission areas where malaria is prevalent year round, adults develop partially protective immunity. Young infants (< 6 months of age) also are afforded some protection, probably from antibodies acquired from their mother, whereas children from 6 months to 5 years of age have the lowest levels of protective immunity and are most susceptible to developing high parasitemia with risks for complications and death (for example, see a study in Kilifi, Kenya[69]). In low-transmission areas or areas that have seasonal malaria, individuals develop lower levels of protective immunity and typically have worse symptomatic malaria upon

infection. This correlation between protective immunity and malaria severity poses a challenge for successful malaria treatment programmes: as the number of infections and transmission rates decrease, increasing numbers of patients will lose protective immunity and become susceptible to severe disease. The re-introduction of malaria in areas that had been malaria-free for many years could be devastating in the short term, and, therefore, well-organized surveillance is required.

[H2] Pathogenesis

The predominant pathogenic mechanism is the haemolysis of *Plasmodium*-infected red blood cells, which release parasites and malaria endotoxin – understood as a complex of hemozoin, parasite DNA and Toll-like receptor 9 (TLR9), a nucleotide-sensing receptor involved in the host immune response against pathogens[70] – that leads to high levels of tumour necrosis factor (TNFα), and clinical symptoms such as fever [71-73] . In addition, the membrane of infected red blood cells becomes stiff, and this loss of deformability contributes to the obstruction of capillaries, with life-threatening consequences in severe malaria when vital organs are affected.[74]

[H3] Parasite factors that influence disease severity. Disease severity and pathogenesis are linked to surface proteins that are expressed by the parasite. In *P. falciparum*, a major surface antigen is encoded by the *var* gene family, which contains ~60 members.[7,11,64,65] The majority of the *var* genes are classified into three subfamilies —A, B and C— based on genomic location and sequence: the B and C groups mediate the binding to host cells via platelet glycoprotein 4 (CD36), whereas the A group genes mediate non-CD36 binding interactions that have been linked to severe malaria, including cerebral malaria[7,65]. The *var* genes encode erythrocyte membrane protein 1 (*Pf*EMP1), with the B and C groups accounting for over 80% of *Pf*EMP1 variants. *Pf*EMP1 is the major protein involved in cytoadherence and mediates the binding of infected erythrocytes to the endothelial vasculature. In cerebral malaria, group A *Pf*EMP1s

mediate binding of infected erythrocytes to endothelial protein C receptor (EPCR) and intercellular adhesion molecule 1 (ICAM-1) in the brain, thereby leading to pathology [8,11,75,76]. However, our knowledge of the host cell receptors that are involved in interactions with the infected erythrocytes is probably incomplete. For example, thrombin, which regulates coagulation via vitamin K-dependent protein C, can cleave *Pf*EMP1, thereby reversing and preventing endothelial binding of infected erythrocytes[75].In pregnancy, the expression of a specific *Pf*EMP1 variant, variant surface antigen 2-CSA (VAR2CSA), which is not encoded by one of the three main subfamilies, leads to an increased risk for placental malaria (Box 3) [7,65]

High parasitemia levels also seem to correlate with poor outcomes[7,76], and the circulating levels of *P. falciparum* histidine-rich protein 2 (encoded by *pfhrp2*) have been used as a biomarker of parasitemia that predicts the risks for microvascular obstruction and severe disease[77]. The brain pathology in children with severe malaria was recently described in detail[78].

P. vivax is thought to cause less-severe disease because it does not have the *var* genes that encode the endothelial binding proteins found in *P. falciparum* and because its ability to only invade reticulocytes leads to lower parasite levels. [7]

[H3] Host traits that influence disease severity. Malaria has exerted a strong selection pressure on the evolution of the human genome [79,80]. Some haemoglobin alleles that in homozygous genotypes cause severe blood disorders (such as thalassemia, the earliest described example, and sickle cell disease) have been positively selected in populations living in malaria endemic areas, because heterozygous genotypes protect against malaria. [81]. Other inherited haemoglobin abnormalities (for example, mutations affecting haemoglobin C and E) can also provide protection against malaria[82].

In addition, genetic polymorphisms that affect proteins expressed by red blood cells and enzyme deficiencies can also be protective against severe disease. The red blood cell Duffy

receptor is a key receptor that mediates invasion of *P. vivax* through interaction with the Duffy binding protein on the parasite surface[47]. Genetic inheritance of Duffy mutations (*Dy-/Dy-)* in Africa is credited with reducing the spread of *P. vivax* in that region, though the finding of Duffy-negative individuals that can be infected with *P. vivax* suggests we still have an incomplete understanding of invasion factors in *P. vivax* [83,84]. Glucose-6-phosphate dehydrogenase (G6PD) deficiency[79,80] provides protection through an unknown mechanism against severe malaria, at least in hemizygous males[85], but unfortunately also leads to haemolytic anaemia in patients treated with primaquine, an 8-aminoquinoline antimalarial and the only agent currently approved for the treatment of latent (liver stage) *P. vivax* malaria. The mode of action of primaquine, a prodrug, remains unknown.

The mechanisms of malaria protection in these varied genetic disorders have been widely studied [82]. Common findings include increased phagocytosis and elimination by the spleen of infected mutant erythrocytes, which reduces parasitemia, reduced parasite invasion of mutant red blood cells, reduced intracellular growth rates, and reduced cytoadherence of infected mutant red blood cells; all these effects increase protection against severe malaria, which is the main driver for human evolution in this case. Some point mutations in the haemoglobin gene alter the display of *Pf*EMP1 on the surface of infected red blood cells, thereby diminishing cytoadherence to endothelial cells [86,87]. This finding highlights the critical role of cytoadherence in promoting severe disease.

Finally, variability in response to TNFα, which is secreted from almost all tissues in response to malaria endotoxins, has also been proposed as a factor mediating differential host responses and contributing to severe malaria when levels are high.[7]

[H1] Diagnosis, screening and prevention

[H2] Diagnosis

The WHO definition of the diagnosis of malaria considers two key aspects of the disease pathology: fever and the presence of parasites.[88] Parasites can be detected with light microscopy examination of a blood smear (Figure 4), or a rapid diagnostic test[88]. The patient's risk of exposure (for example, the patient lives in an endemic region, or his or her travel history might indicate exposure) can assist in making the diagnosis. Furthermore, clinical expression of *Plasmodium* infection correlates with the level of transmission in the area. Symptoms of uncomplicated malaria include sustained episodes of high fever (Box 1); when high levels of parasitaemia are reached, several life-threatening complications might occur (severe malaria) (Box 1).

Complications in severe malaria mostly relate to infected red blood cells blocking blood vessels, with severity and symptoms depending on what organ is affected (Box 1) and with what intensity, and differ by age: lungs and kidney disease is unusual in children in Africa, but common in non-immune adults.

[H3] Parasitaemia. Patients with uncomplicated malaria typically have parasitaemia in the range of 1,000-50,000 per microliter (however, parasite densities below 1,000 can also present symptoms in non-immune travellers and young children). The higher densities tend to be associated with severe malaria, but the correlation is imprecise and there is no cut-off density. In a pooled analysis of patient data from 61 studies that were designed to measure the efficacy of ACTs (throughout 1998 - 2012), parasitaemia averaged ~4,000 per microliter in South America, ~10,000 per microliter in Asia and ~20,000 per microliter in Africa[89]. The limit of detection by thick smear microscopy is ~50 parasites per microliter.[90] WHO-validated rapid diagnostic tests can detect 50 to 1,000 parasites per microliter with high specificity, but many lack sensitivity, especially as compared to PCR-based methods[91]. The ability to detect low levels of parasitaemia is important to predict clinical relapses, as parasitaemia can increase 20-

fold over a 48 hour cycle period. These data are based on measurements in healthy volunteers (Controlled Human Infection models) who were infected at a defined time point with a known number or parasites, and in whom the asymptomatic parasite reproduction was monitored by qPCR up to the point the individual received rescue treatment[92].

In hyperendemic areas (with all-year disease transmission), often many children and adults are asymptomatic carriers of the parasite. In these individuals, the immune system maintains parasites at equilibrium levels in a tug-of-war. However, parasitaemia in asymptomatic carriers can be extremely high, with reports of levels as high as 50,000 per microliter in a study of asymptomatic pregnant women, (range 80- 55,400/μl) [93]. In addition to the obvious risks for such people, they represent a reservoir for infecting mosquitoes, leading to continued transmission. In clinical studies, the parasitaemia of asymptomatic carriers can be monitored with PCR-based methods, which can detect as low as 22 parasites per millilitre.[94] However, detection of low-level parasitaemia in low-resource settings requires advanced technology. Loop-mediated isothermal amplification (LAMP[95]) is one promising approach. This type of PCR is fast (10^9-fold amplification in an hour) and does not require thermal cycling, reducing the requirement for expensive hardware. Versions of this method that do not require electricity are being developed[96]. Nucleic acid-based techniques such as LAMP and PCR-based methods also have the advantage that they can be used to detect multiple pathogens simultaneously, and, in theory, identify drug-resistant strains[97]. This approach enables accurate diagnosis of which *Plasmodium* species is involved, and in the future could lead to the development of multiplexed diagnostics that enable differential diagnosis of the causative pathogens (including bacteria and viruses) in patients who present with fever[98].

[H3] Rapid diagnostic tests. Rapid diagnostic tests are based on the immunological detection of parasite antigens (lactate dehydrogenase (LDH) or histidine-rich protein) in the blood, have sensitivities comparable with that of light microscopy examination but have the

advantage that they do not require extensive training of the user. These tests provide rapid diagnosis at point-of-care level in resource-limited settings, and can therefore substantially improve malaria control. However, occasionally, false positive results from rapid diagnostic tests can be problematic, because they could lead to the wrong perception that antimalarial medicines are ineffective. False-negative test results have been reportedly caused by *pfhrp2* gene deletions in *P. falciparum* strains in South America [99-104]. Current data suggest that LDH-targeting rapid diagnostic tests are less sensitive for *P. vivax* than for *P. falciparum*[105], and limited information on the sensitivity of these tests for the rarer species, such as *P. ovale* or *P. malariae*, is available. Rapid diagnostic tests also offer great possibilities in tracking malaria epidemiology: photos of the results of the tests taken with mobile phones can be uploaded to databases (even using cloud-based data architecture[106]) and provide an automated collection of surveillance data[107].

[H2] Prevention in vulnerable populations

Prevention of *Plasmodium* infection can be accomplished by different means: vector control, chemoprevention and vaccines. Mosquito (vector) control methods include (from the broadest to the most targeted: the widespread use of insecticides, such as in the 1960s DDT campaigns, the destruction of breeding grounds (that is, draining marshes and other breeding reservoirs), indoor residual spraying with insecticides (that is, the application of residual insecticide inside dwellings, on walls, curtains or other surfaces), the use of larvicides and the use of insecticide-treated bed nets. The use of endectocides has also been proposed: these drugs, such as ivermectin, kill or reduce the lifespan of mosquitoes which feed on individuals who have taken them [108]. However, this approach is still experimental: individuals would be taking drugs with no direct benefit for themselves (as they do not directly prevent human illness), and so the level of safety data required for registration of endectocides will need to be

substantial. Vector control approaches differ in efficacy, costs and the extent of their effect on the environment. Targeted approaches such as insecticide-treated bed nets have had a strong effect. Chemoprevention is an effective strategy that has been employed to reduce malaria incidence in campaigns of seasonal malaria chemoprevention, in intermittent preventative treatment for children and pregnant women, and for mass drug administration [109]. Such antimalarials need to have an excellent safety profile since they are given to large numbers of healthy people. Vaccines excel in eradicating disease, but effective malaria vaccines are challenging because, unlike viruses and bacteria against which effective vaccines have been developed, protists pathogens (like *Plasmodium*), are large-genome microorganisms that have evolved highly effective immune evasion strategies (such as encoding dozens or hundreds of cell surface protein variants). Nevertheless, the improved biotechnological arsenal to generate antigens and improved adjuvants could help to overcome such issues.

[H3] Vector control measures. The eradication of mosquitoes is no longer considered an option to eliminate malaria; however, changing the capacity of the vector reservoir has substantial effects on malaria incidence: long-lasting insecticide-treated bed nets and indoor residual spraying have been calculated to be responsible for two-thirds of the malaria cases averted in Africa between 2000 and 2015 (Ref. [12]). Today's favoured and more-focused vector-control approach involves the use of fine-mazed, sturdy, long-lasting and wash-proof insecticide-treated bed-nets.[110] The fabric of these nets is impregnated with an insecticide that maintains its efficacy after at least 20 standardized lab washes and have a three year recommended use. Insects are attracted by the person below the net, but are killed as they touch it. However, the efficacy of bed nets is threatened by several factors, including inappropriate use of the nets (for example, for fishing purposes) and behavioural changes in the mosquitoes, which have begun to bite also during the da[111]. The main problem, however, is the increasing emergence of vector resistance to insecticides, especially pyrethroids[111] and,

429 therefore, new insecticides with different modes of action are urgently needed. New insecticides
430 have been identified by screening millions of compounds from the libraries of agrochemical
431 companies, but even those at the most advanced stages of development are still 5-7 years from
432 deployment (Figure 5)[112,113]. Few of these new insecticides are suitable for application in bed
433 nets (because of high costs, or unfavourable chemical properties) but some can be used for
434 indoor residual spraying. New ways of deploying these molecules are also being developed,
435 such as improved spraying technologies[114], timed release to coincide with seasonal
436 transmission and slow-release polymer-based wall linings[115,116].

437 Genetic approaches, fuelled by advances in the CRISPR-Cas9 gene editing technology,
438 represent an exciting area of development for novel insect control strategies. There are
439 currently two main approaches: population suppression, whereby mosquitoes are modified so
440 that any progeny are sterile, and population alteration, whereby mosquitoes are modified so that
441 progeny are refractory to *Plasmodium* infection[117,118]. Initial approaches to population
442 suppression involved releasing sterile male insects[119]. These strategies have now been
443 developed further, with the release of male insects carrying a dominant lethal gene, which kills
444 their progeny[120,121]. Gene drive systems can be used for both population suppression and
445 population alteration. These systems use homing endonucleases, which are microbial enzymes
446 that induce lateral transfer of an intervening DNA sequence and can, therefore, convert a
447 heterozygote into a homozygote. Homing endonucleases have been re-engineered to recognise
448 mosquito genes[122], and can rapidly increase the frequency of desirable traits in a mosquito
449 population[123]. Gene drive has now been used in feasibility studies to reduce mosquito
450 populations[124], or make them less able to transmit malaria parasites[125]. Another approach is
451 inspired by the finding that *Aedes aegypti* mosquitoes (the vector for Dengue, Yellow Fever and
452 Zika viruses) infected with bacteria of the *Wolbachia* species (a parasite that naturally colonizes
453 numerous species of insects) cannot transmit the Dengue virus to human hosts[126]. Symbiont

454 *Wolbachia* can be modified to make them deleterious to other parasites in the same host, and
455 progress has been made in finding symbionts that can colonise *Anopheles* mosquitoes[127,128].
456 Although all the above approaches are very promising, they are still at a very early stage, and
457 the environmental uncertainties associated with widespread distribution of such technologies, as
458 well as the complex regulatory requirements, provide additional hurdles that will need to be
459 overcome.

460 **[H3] Chemoprotection and chemoprevention.** Chemoprotection describes the use of
461 medicines (given at prophylactic doses) to temporarily protect subjects entering an area of high
462 endemicity, historically tourists and military personnel, and populations at risk from emergent
463 epidemics, but is also being increasingly considered for individuals visiting areas that have
464 become recently malaria free. Chemoprevention, often used in the context of seasonal malaria,
465 describes the use of medicines with demonstrated efficacy for treatment that are given regularly
466 to large populations who live in areas of high endemicity at full treatment doses (as some of the
467 individuals treated will be asymptomatic carriers).

468 Currently there are three 'gold standard' drugs for chemoprotection: atovaquone-
469 proguanil, doxycycline (both of which require daily doses), and mefloquine, which is taken
470 weekly. Mefloquine is the current mainstay against the spread of multidrug-resistant
471 *Plasmodium* in the Greater Mekong Sub-region of South East Asia, despite having a black box
472 warning for psychiatric adverse events; however, an analysis of pooled data from 20,000 well-
473 studied patients found this risk was small (fewer than 12 cases per 10,000 treatments).[129] An
474 active search to find new medicines that could be useful in chemoprotection, in particular
475 medicines that can be given weekly or even less frequently is underway. One interesting
476 possibility is long-acting injectable intra-muscular combination chemoprotectants, which if
477 effective could easily compete with vaccination, if they provided protection with 3-4 injections
478 per year. Such an approach (called pre-exposure prophylaxis) is being studied for HIV (which

479 also poses major challenges in the development of an effective vaccine)[130], and may lead to the
480 development of long-acting injectable drug formulations[131] produced as crystalline nanoparticles
481 (to enhance water-solubility) using the milling technique.

482 Chemoprevention generally refers to seasonal malaria chemoprevention campaigns,
483 which target children <5 years of age[132]. In the Sahel region (the area just south of the Sahara
484 desert, where there are seasonal rains and a recurrent threat of malaria), seasonal malaria
485 chemoprevention with a combination of sulfadoxine-pyrimethamine plus amodiaquine had a
486 strong effect[133-137], with a reduction of malaria cases of >80% among children and a reduction of
487 mortality of >50%[138]. Although these campaigns are operationally complex – the treatment has
488 to be given monthly – between 2015 and 2016 over 20 million children have been protected, at
489 a cost of ~US$1 per treatment. A concern about seasonal malaria chemoprevention is the
490 potential for a rebound effect of the disease. Rebound could occur if children lose immunity
491 against malaria while receiving treatment that is later stopped because they reached the age
492 limit, if campaigns are interrupted because of economic difficulties or social unrest (war) or if
493 drug resistance develops. Because of the presence of resistant strains, a different approach is
494 needed in African areas south of the Equator[139], which led to trials of monthly three-day courses
495 of ACTs in seasonal chemoprevention[137]; there is growing literature on the impressive efficacy
496 of dihydroartemisinin (DHA)-piperaquine to prevent malaria in high risk groups.[140] To reduce the
497 potential for the emergence of drug resistance, the WHO good practice standards state that,
498 when possible, drugs used for chemoprevention should differ from the front-line treatment that is
499 used in the same country or region [109], underscoring the need for the development of multiple,
500 new and diverse treatments to provide a wider range of options.

501 Finally, intermittent preventive treatment is also recommended to protect pregnant
502 women in all malaria-endemic areas (Box 3).[109]

[H3] Vaccines. Malaria, along with tuberculosis and HIV infection, is a disease in which all components of the immune response (both cellular, in particular during the liver stage, and humoral, during the blood stage) are involved, and this means that developing an effective vaccine will be a challenge. The fact that adults living in high-transmission malarious areas acquire partial protective immunity indicates that vaccination is a possibility. As a consequence, parasite proteins targeted by natural immunity, such as the circumsporozoite protein (the most prominent surface antigen expressed by sporozoites), proteins expressed by merozoites and parasite antigens exposed on the surface of infected red blood cells[141] have been studied for their potential to be used in vaccine programs[142]. However, experimental malaria vaccines tend to target specific parasite species and surface proteins, an approach that both restricts their use and provides scope for the emergence of resistance. Sustained exposure to malaria is needed to maintain natural protective immunity, which is otherwise lost in 3-5 years[143], perhaps as a result of clearance of circulating antibodies and failure of memory B cells to develop into long-lived plasma B cells. Controlled Human Infection models[144-146] have started to provide a more-precise understanding of the early cytokine and T-cell responses in naïve subjects, underscoring the role of the regulatory T-cells in damping the response against the parasite, resulting in an exhaustion of T cells[147]. Vaccine development is currently focusing on using multiple antigens from different stages of the parasite lifecycle. Future work will also need to focus on the nature of the immune response in man, and specifically the factors leading to diminished T-cell responses. New generations of adjuvants are needed, possibly compounds that produce the desired specific response, rather than a general immune stimulation. This is a challenging area of research, as adjuvants have often completely different efficacy in humans and preclinical animal models.

Currently there is no licenced vaccine against malaria. The ideal vaccine should protect against both *P. falciparum* and *P. vivax*, with a protective, lasting efficacy of at least 75%. The

most advanced candidate is RTS,S (trade name Mosquirix, developed by GlaxoSmithKline and the PATH-Malaria Vaccine Initiative), which contains a recombinant protein with parts of the *P. falciparum* circumsporozoite protein combined with the hepatitis B virus surface antigen, with a proprietary adjuvant. RTS,S reduced the number of malaria cases by half in 4,358 children 5–17 months of age during the first year following vaccination[148], preventing 1,774 cases for every 1,000 children thanks to herd immunity, and had an efficacy of 40% over the entire 48 months of follow-up in children that received four vaccine doses over a four-year period[149]. Efficacy during the entire follow-up dropped to 26% when children only received three vaccine doses. Efficacy during the first year in 6-12 week old children was limited to 33%. Thus, the RTS,S vaccine fails to provide long-term protection. Further studies, as requested by the WHO, will be done in pilot implementations of 720,000 children in Ghana, Kenya and Malawi (240,000 each, half of which will receive the vaccine), before a final policy recommendation is made. However, a vaccine with only partial and short-term efficacy could still be used in the fight against malaria. RTS,S could be combined with chemoprevention to interrupt malaria transmission in low-endemic areas.[150] Thus, vaccines unable to prevent *Plasmodium* infection could be used to prevent transmission (for example, by targeting gametocytes), or as additional protective measure for pregnant women.

A large pipeline of vaccine candidates is under evaluation (Figure 6). These include irradiated sporozoites, an approach that maximizes the variety of antigens exposed[151], and subunit vaccines, which could be developed into multi-component, multi-stage and multi-antigen formulations[152]. Although vaccines are typically designed for children, as the malaria map shrinks, both paediatric and adult populations living in newly malaria-free zones will need protection, because they would probably be losing any naturally acquired immunity and, therefore, be more-susceptible. Indeed, in recent years there has been a focus on transmission-blocking vaccines to drive malaria elimination. This approach has been labelled altruistic, as

vaccination would have no direct benefit for the person receiving it, but it would benefit the community; a regulatory pathway for such a novel approach has been proposed[153,154]. The most clinically advanced vaccine candidate based on this approach is a conjugate vaccine the targets the female gametocyte marker Pfs25 (Ref. [155]), and other antigens are being tested pre-clinically. Monoclonal antibodies are another potential tool to provide protection. Improvements in manufacturing and high-expressing cell lines are helping to overcome the major barrier to their use (high costs)[156], and improvements in potency and pharmacokinetics are reducing the volume and frequency of administration [157]. Monoclonal antibodies could be particularly useful to safely provide the relatively short-term protection needed in pregnancy. The molecular basis of the interaction between parasites and placenta is quite well understood; two Phase I trials of vaccines that are based on the VAR2CSA antigen are under way[158,159].

[H1] Management

No single drug is effective against all *Plasmodium* species or all of the manifestations of the disease that occur in different patient populations. Thus, treatment must be tailored to each situation appropriately[109,160]. Firstly, the treatments of uncomplicated and severe malaria are distinct. In uncomplicated malaria, the treatment of choice is an oral medicine with a low adverse effect profile. However, in severe malaria, the preferred initial therapy includes parenteral administration of an artemisinin derivative, as this formulation has a quick onset and can rapidly clear the parasites from the blood, and is also suitable for those patients with changes in mental status (such as coma) that make swallowing oral medications impossible. For treatment of malaria in pregnancy, the options are limited to the drugs that are known to be safe for both expectant mother and foetus, and different regimens are needed (box 2). Different drugs are used for different *Plasmodium* species, a choice usually driven more by drug resistance frequencies (lower in *P. vivax, P. ovale, P. malariae* and *P. knowlesi* compared with *P. falciparum*) rather than by species differences as such. Thus, chloroquine, with its low cost

578 and excellent safety, is used in most cases of non-falciparum malaria, where it remains
579 effective, whereas falciparum malaria requires newer medicines that overcome resistance
580 issues. The persistence of *P. vivax* and *P. ovale* hypnozoites, even after clearance of the stages
581 that cause symptoms, necessitates additional treatments. Only primaquine targets hypnozoites.

582 **[H2] *P. falciparum* malaria**

583 The mainstay treatments for uncomplicated *P. falciparum* malaria are ACTs: fixed-dose
584 combinations of two drugs, an artemisinin derivative and a quinine derivative[109]. (Table 1, box
585 4).

586 Because of its high lipophilicity, artemisinin itself is not the molecule of choice in any
587 Stringent Regulatory Authorities -approved combination. Instead, semi-synthetic derivatives are
588 used, either DHA (the reduced hemiacetal of the major active metabolite of many artemisinins),
589 artesunate (a succinate prodrug of DHA, which is highly water soluble) or artemether (a
590 methylether prodrug of DHA).

591 Quinine has been used in medicine for centuries[161], but it was only in the 20th century
592 that a synthetic form was made, and the emerging pharmaceutical and government research
593 sectors delivered the next generation medicines that built on it. The combination partners of
594 choice are 4-aminoquinolines (for example, amodiaquine, piperaquine and pyronaridine) and
595 amino-alcohols (such as mefloquine or lumefantrine); these molecules are believed to interfere
596 with hemozoin formation. There are now five ACTs that have been approved or are close to
597 approval by the FDA, EMA or WHO Prequalification (Table 1 and Figures 7 and 8). In pivotal
598 clinical studies, these combinations have proven extremely effective (adequate clinical and
599 parasitological response (that is, absence of parasitaemia at day 28) >94%, see for example
600 Ref [162]) , are well tolerated (as they has been given to over 300 million paediatric patients),

affordable (typically under US $1 per dose) and, thanks to ingenious formulations and packaging, stable in tropical climate conditions.

Following the results of comprehensive studies in Africa and Asia, the injectable treatment of choice for severe falciparum malaria is artesunate[163-165]. In the United States, artesunate for intravenous use is available as an Investigational New Drug (IND) through the CDC (Centers for Disease Control and Prevention) malaria hotline and shows efficacies of above 90% even in patients who are already unconscious[166]. Sometimes, however, in low-income countries it is necessary to administer intravenous quinine or quinine while awaiting an artesunate supply. Suppositories of artesunate are in late stage product development[167], and already available in Africa, as a pre-referral treatment to keep patients alive while they reach a health clinic.

[H2] *P. vivax* malaria

Chloroquine or ACTs are WHO-recommended for uncomplicated vivax malaria[109] (although chloroquine is no longer used in several countries, for example, Indonesia). Since chloroquine-resistant *P. vivax* is becoming increasingly widespread, particularly in Asia, the use of ACTs is increasing; although only artesunate-pyronaridine is approved for the treatment of blood stage *P. vivax* malaria, the other ACTs are also effective, and are used off-label. Relapses of *P. vivax* malaria present a problem in malaria control. Relapse frequencies differ among *P. vivax* strains: they are high (typically within three weeks) in all-year transmission areas, such as Papua New Guinea, but relapse occurs on average after seven months in areas with a dry or winter season. Some *P. vivax* strains, such as the Moscow and North Korea strains, are not, in most cases, symptomatic at the time of first infection, but become symptomatic only on reactivation of the hypnozoites. [168] Primaquine needs to be administered in addition to the primary treatment to prevent relapse and transmission, which can occur even years after the primary infection. Primaquine treatment, however, lasts 14 days, has gastro-

626 intestinal adverse effects in some patients and is contra-indicated in pregnant women and in
627 patients who are deficient or express low levels of G6PD (as it can cause haemolysis).
628 Tafenoquine[169], a next generation 8-aminoquinoline, is currently completing Phase III clinical
629 studies. As with primaquine, patients will still require an assessment of their G6PD enzyme
630 activity for safe use to determine the optimal dose. In phase II studies, tafenoquine was shown
631 to have similar efficacy as primaquine, but with a single dose only compared with the 7-14 day
632 treatment with primaquine; higher patient compliance is expected to be a major benefit of a
633 single-dose regimen. The ultimate elimination of *P. vivax* malaria will be dependent on the
634 availability of safe and effective anti-relapse agents and is, therefore, a major focus of the drug
635 discovery community.

636 **[H2] Drug resistance**

637 The two drugs that compose ACTs have very different pharmacokinetic profiles in
638 patients. The artemisinin components have a plasma half-life of only a few hours, yet can
639 reduce parasitaemia by 3-4 orders of magnitude. On the other hand, the 4-aminoquinolines or
640 amino-alcohols have long (>4 days) terminal half-lives, providing cure (defined as adequate
641 clinical and parasitological response) and varying levels of post-treatment prophylaxis. The
642 prolonged half-life of the non-artemisinin component of ACTs has raised concerns in the
643 research community, owing to the risk of drug resistance development. However, the
644 effectiveness of the ACTs in rapidly reducing parasitaemia suggests that any emerging
645 resistance has arisen largely as a result of poor clinical practice: the use of artemisinins as
646 monotherapy, lack of patient compliance and sub-standard medicine quality (including
647 counterfeits) — all situations in which large numbers of parasites are exposed to a single active
648 molecule [170]. However, partial resistance to piperaquine[171] and artemisinin [172] (which manifests
649 as a reduced rate of parasite clearance rate rather than a shift in IC_{50}) has been confirmed in
650 the Greater Mekong Subregion, as well as resistance to mefloquine and amodiaquine in various

parts of the world[173]. Africa has so far been spared, but reports of either artemisinin [174] or ACT treatment failures [175] in African isolates of *P. falciparum* have raised concerns. Thus, artemisinin-resistant *Plasmodium* and insecticide-resistant mosquitoes are major threats to the progress that has been made in reducing malaria deaths through the current control programs. It is important to emphasize that progress against malaria has historically been volatile; in many areas the disease re-emerged as the efficacy of old drugs was lost in strains that developed resistance.

Large strides have been made towards identifying genetic markers in *Plasmodium* that correlate with resistance to clinically used drugs (Table 2). These markers enable the research and medical community to proactively survey parasite populations to make informed treatment choices. Cross-resistance profiles reveal reciprocity between 4-aminoquinolines and amino-alcohols (parasites resistant to one class are more sensitive to the other). Additionally a drug can exert two opposite selective pressures, one towards the selection of resistant mutant and the other towards the selection of strains with increased sensitivity to a different drug, a phenomenon known as "inverse selective pressure"[176,177]. These findings support the introduction of treatment rotation or triple combination therapies as potential future options. Finally, the drug discovery and development pipeline is delivering not only new compounds that have novel modes of action and overcome known resistant strains, but also chemicals with the potential to be effective in a single dose, to overcome compliance issues. Nevertheless, policymakers need to be on high alert to prevent or rapidly eliminate outbreaks of resistant strains and to prioritize the development of new treatments.

[H2] Drug discovery and development pipeline

The most comprehensive antimalarial Discovery portfolio has been developed by the not-for-profit PDP Medicines for Malaria Venture (MMV) in collaboration with its partners in both academia and the pharmaceutical industry, with generous support from donors (mainly

676 government agencies and philanthropic foundations). (Figure 7). Promising compound series
677 have been identified from three approaches: hypothesis-driven design to develop alternatives to
678 marketed compounds (for example, synthetic peroxides such as ozonides), target-based
679 screening and rational design (for example, screening of inhibitors of P. falciparum
680 dihydroorotate dehydrogenase (DHODH)) and phenotypic screening[178]. Phenotypic screening is
681 the most successful approach to date, in terms of delivering preclinical candidates and
682 identifying, through sequencing of resistant mutants, novel molecular targets. However, with the
683 advances in the understanding of parasite biology and in molecular biology technology, target-
684 based approaches will probably have a substantial role in the coming years.

685 Two combinations, OZ439-Ferroquine (Sanofi and MMV) and KAF156-Lumefantrine
686 (Novartis and MMV), are gearing up to begin Phase IIb development to test the efficacy of
687 single dose cure and, in the case of KAF156-Lumefantrine, additionally two- or three-day cures.
688 OZ439, or artefenomel, is a fully synthetic peroxide with sustained plasma exposure from a
689 single, oral dose in humans[179,180]; the hope is that it could replace the three independent doses
690 required with an artemisinin derivative. Sanofi's ferroquine is a next generation 4-
691 aminoquinoline without cross-resistance to chloroquine, amodiaquine or piperaquine[181,182].
692 KAF156 is a novel imidazolopiperazine with unknown mechanism of action[183-185], but its
693 resistance marker, *P. falciparum* Cyclic Amine Resistance Locus (*PfCARL*), appears to code for
694 a transporter on the endoplasmic reticulum membrane of the parasite. Interestingly, whilst
695 OZ439 and ferroquine principally affect asexual blood stages, KAF156 also targets both the
696 asexual liver stage and the sexual gametocyte stage and, therefore, could have an effect on
697 transmission.

698 Two other compounds, KAE609 (also known as cipargamin[186,187]) and DSM265[188-191],
699 are poised to begin Phase IIb and are awaiting decisions on combination partners. KAE609 is a
700 highly potent spiroindolone that provides parasite clearance in patients even more rapidly than

peroxides; its assumed mode of action is the inhibition of *Pf*ATP4 (Figure 3) (encoded by its resistance marker), a transporter on the parasite plasma membrane that regulates Na^+/proton homeostasis. Inhibition of this channel, identified through sequencing of resistant mutants, increases Na^+ concentration and pH, which results in parasite swelling, rigidity and fragility that contribute to host parasite clearance in the spleen on top of intrinsic parasite killing. In addition, effects on cholesterol levels in the parasite plasma membrane have been noted that are also likely to contribute to parasite killing by leading to increased rigidity that results in more rapid clearance *in vivo* [192]. DSM265 is a novel triazolopyrimidine with both blood and liver stage activity that that selectively inhibits the *Plasmodium* enzyme PfDHODH (Figure 3). It was optimized for drug-like qualities from a compound that was identified from a high throughput screen of a small molecule library[189,193]. DSM265 maintains a serum concentration above its minimum parasiticidal concentration in humans for 8 days, and had efficacy in both treatment and chemoprevention models in human volunteers in Phase Ib trials[188,191].

Within Phase I, new compounds are first assessed for safety and pharmacokinetics, and then for efficacy against asexual blood or liver stages of *Plasmodium* using a controlled human malaria infection model in healthy volunteers[146]. This model provides a rapid and cost-effective early proof of principle and, by modelling the concentration-response correlation, increases the accuracy of dose predictions for further clinical studies. The 2-aminopyridine MMV048 (MMV390048, Refs.[194,195]), (+)-SJ733 (SJ557733; Refs. [58,196]) and P218 (Ref.[197]) are currently progressing through Phase I. MMV048, inhibits *Pf*PI(4)K, (Figure 3) and this inhibition affects the asexual liver and blood stages as well as the sexual gametocyte stage. MMV048 has good exposure in animal models[195], suggesting it could potentially be used in a single dose use in combination with another drug. SJ733, a dihydroisoquinolone, inhibits PfATP4 and is an alternative partner with a completely different structure from KAE609 that has excellent

725 preclinical safety and development potential. P218 is currently being evaluated for testing in the
726 controlled human malaria infection cohort.

727 A further eight compounds are undergoing active preclinical development [198]. Of these
728 compounds, four are alternatives to the leading compounds that target established
729 mechanisms: PA92 (PA-21A092, Ref. [199]) – an aminopyrazole – and GSK030 (GSK3212030A)
730 – a thiotriazole – both target PfATP4, DSM421[200] is a triazolopyrimidine alternative to DSM265
731 and UCT943 (MMV642943)[201] is an alternative to MMV048. Three compounds show novel
732 mechanisms of action or resistance markers: DDD498 (DDD107498[202]) inhibits *P. falciparum*
733 elongation factor 2 (and, therefore, protein synthesis) and has outstanding efficacy against all
734 parasite lifecycle stages, MMV253 (AZ13721412)[203] is a fast-acting triaminopyrimidine with a V-
735 type ATPase as resistance marker and AN762 (AN13762) is a novel oxaborole [204] with a novel
736 resistance marker. All these compounds are developed by collaborations with MMV.

737 The eighth compound in active preclinical development, led by Jacobus Pharmaceuticals, is
738 JPC3210[205], a novel aminocresol that improves upon the historical candidate, WR194965,
739 which was developed by the Walter Reed Army Institute of Research and tested in patients at
740 the time of the development of mefloquine in the 1970s. JPC3210 has an unknown mechanism
741 of action with potent, long-lasting efficacy in preclinical models, suggesting the potential to be
742 used in a single dose for both treatment and prophylaxis[205].

743 **[H1] Quality of life**

744 Malaria is one among the diseases of poverty. On the WHO web-site it is stated: "*There*
745 *is general agreement that poverty not only increases the risk of ill health and vulnerability of*
746 *people, it also has serious implications for the delivery of effective health-care such as reduced*
747 *demand for services, lack of continuity or compliance in medical treatment, and increased*
748 *transmission of infectious diseases*[206]." The socio-economic burden of malaria is enormous and

749 although the disease prevalently affects children, it is a serious obstacle to development and
750 economy[207]. Malaria is responsible for annual expenses of well over billions of euros in some
751 African countries[208]. In many endemic areas, each individual suffers multiple episodes of
752 malaria per year, each causing loss of school time for children and work time for their parents
753 and guardians. Despite the declining trends in malaria morbidity and mortality, the figures are
754 still disconcertingly high for a disease that is entirely preventable and treatable[16].

755 Malaria has long-term detrimental effects also on non-health-related quality of life of the
756 affected population: it intensifies poverty by limiting education opportunities, as it leads to
757 absenteeism in schools and reduced productivity at work[16]. The effects of acute illness normally
758 drive families to seek urgent attention, which may consist of self-medication, if the disease is
759 familiar to the household. Yet even an episode of uncomplicated malaria can potentially be fatal,
760 owing to delay in prompt access to efficacious antimalarial drugs. Because malaria is so familiar
761 to many households, patients, especially children, may be presented late for early diagnosis and
762 treatment in health facilities. Late presentation prolongs morbidity, increases the risk for severe
763 malaria and deprives the families of income through direct expenses and reduced productivity.
764 Frequent disease episodes experienced in the endemic areas as well as their possible
765 complications can negatively affect child growth and nutrition, shortening the lives of children
766 and family members. The neurological consequences can affect a child's ability to learn and
767 become a self-reliant adult[209-211], as they often occur at an important growth phase of the brain,
768 when areas involved in higher learning (such as planning, decision-making, self-awareness and
769 social sensitivity) mature. Cognitive deficits occurring during the early education years affect the
770 entire family, as they impair the child's ability to contribute to the well-being of the family as they
771 grow and put additional strain on the parents, who may sometimes have to care for a
772 substantially disabled child and, later, an adult[212].

773

[H1] Outlook

The agenda set by the WHO aims for malaria incidence and mortality to decrease by 90% over the next 15 years, with increasing numbers of countries that eliminate the disease[213] Even if we achieve the ambitious goals set by the WHO, there will still be a child dying of malaria every 10 minutes in 2030. The ACTs are extraordinarily effective, and much of the disease burden could be reduced by complete deployment and availability of these medicines. There are now two approved ATCs that are specifically designed (taste-masked and sweetened) for paediatric use.

However, the emergence of drug-resistant *Plasmodium* and insecticide-resistant mosquitoes is a major concern. The first clinical reports of artemisinin resistance appeared from the Thai-Cambodia border region in the mid-2000s[214]. So far, resistant strains have not spread to Africa, and the severity of the malaria caused by artemisinin-resistant parasites is not different from that of disease caused by wild type strains. However, if artemisinins became ineffective, no alternative first-line treatments would be available, as new therapies are still only in phase II clinical trials and their safety and efficacy will need to be effectively assessed in the field before they can be deployed for wide-spread clinical use.

[H2] Diagnostics

Future diagnostics should address two main issues. Ideally, new diagnostic tests would be non-invasive and not require a blood sample. Many approaches have been piloted, including parasite antigen detection in saliva[215] or urine[216], detection of specific volatile chemical in breath[217] and direct, non-invasive measurements of iron-rich hemozoin in skin blood vessels[218]. Secondly, diagnostics should to be able to detect drug-resistant strains directly in the point-of-care setting, rather than in sentinel sites, to provide better treatment and generate more-detailed epidemiologic maps[219]. A next-generation amplicon sequencing method suitable for use in

798 endemic countries would enable high-throughput detection of genetic mutations in six *P.*
799 *falciparum* genes associated with resistance to anti-malarial drugs, including ACTs, chloroquine
800 and sulfadoxine-pyrimethamine[220].

801 **[H2] Malaria challenges**

802 Besides the length of the process of discovery and development of new drugs,
803 insecticides and vaccines, in malaria there is the additional hurdle of delivery of these new
804 compounds, which first need to obtain approval from all local regulatory authorities. There is a
805 trend for harmonization of the approval requirements among different authorities, with an
806 initiative involving several regional African organizations, for example, to review data on behalf
807 of many countries, similarly to the European Medicines Agency reviewing files on behalf of all
808 the EU countries. These events are paving the way to shorten the time from the end of clinical
809 studies to the day of large-scale deployment, when affected populations will start to reap the
810 benefits .

811 **[H2] The move towards elimination**

812 High-content cellular assays are available to test inhibitors of transmission and
813 compounds that target hypnozoites[221,222]. Discovery efforts for treatment and chemoprotection
814 combinations conform to the malaria Target Product Profiles, a planning tool for therapeutic
815 candidates based on FDA guidelines, to ensure that what is delivered has clinical relevance.
816 The MMV has defined[223] and updated[224] Target Candidate Profiles (TCPs), which define the
817 attributes that are required for the ideal medicines and have proven invaluable in guiding single
818 molecule optimization and decision making.

819 The current focus is moving beyond TCP1 (that includes molecules that clear asexual
820 blood stage parasitemia) – the goal is to deliver compounds that do not simply treat patients and
821 control symptoms but have biological activity that disrupts the lifecycle of the parasite and hence

822 break the transmission cycle, a step that is necessary in the move towards elimination.
823 Particular areas of interest are new compounds for chemoprotection with liver stage activity
824 (TCP2), anti-relapse agents for vivax malaria (TCP3, compounds that target hypnozoites),
825 gametocytocidal compounds to block transmission (TCP5) and compounds that kill hepatic
826 schizonts (TCP4) and protect from the onset of symptomatic stages. Future projects include
827 long-lasting endectocides (TCP6) such as ivermectin[108]. The MMV Discovery portfolio also
828 includes alternative compounds to the clinical frontrunners, molecules with new mechanisms of
829 action (which target, for example, as N-myristoyltransferase[225], Coenzyme A biosynthesis[226],
830 phenyalaninyl[227] and prolyl[228] tRNA synthetase, plasmepsin V[229] and the Qi site of cytochrome
831 bc1 [230]) and compounds that appear resistance-proof (at least *in vitro*).

832

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Author contributions

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Box 1. Malaria key terms

- Asymptomatic malaria: can be caused by all *Plasmodium* species; the patient has circulating parasites but no symptoms.
- Uncomplicated malaria: can be caused by all *Plasmodium* species. Symptoms are non-specific and can include fever, moderate to severe shaking chills, profuse sweating, headache, nausea, vomiting, diarrhoea and anaemia, with no clinical or laboratory findings of severe organ dysfunction
- Severe (complicated) malaria: usually caused by infection with *Plasmodium falciparum*, though less frequently can also be caused by *Plasmodium vivax* or *Plasmodium knowlesi*. Complications include severe anaemia and end-organ damage, including coma (cerebral malaria), pulmonary complications (for example, oedema and hyperpnoeic syndrome[231]) and hypoglycaemia or acute kidney injury. Severe malaria is often associated with hyperparasitaemia and is associated with increased mortality.
- Placental malaria – parasites are present in the placenta, leading to poor outcomes for the foetus and possibly the mother.

Box 2. *Plasmodium* genome and genomic tools for understanding gene function

[H1] Characteristics of *Plasmodium* genome

- Each haploid genome consists of 23 megabases, which encode the program for the parasites' complex life cycle within ~5,500 genes[17-19].
- Many genes encode proteins that have similarities to host proteins, many are novel and many (about half) remain annotated as hypothetical or of unknown function.
- Plasmodium genome includes an essential plastid, the apicoplast, which is derived from two sequential endosymbiotic events and encodes genes from both plant (red algal) and bacterial (cyanobacterium) origin[232]. The bacterial origin of some enzymes encoded by the plastid make *Plasmodium* sensitive to some antibacterial agents while the plant-like pathways can be targeted by herbicides. This plastid is one source of genes that differ from the host and have been considered as potential drug targets.
- Gene transcription across the *Plasmodium* intraerythrocytic lifecycle follows a pre-programmed cyclic cascade where most genes are expressed at peak levels only once per life cycle[233-235]. Genes encoding cell surface proteins involved in host-parasite interactions are the exception.
- Gene expression patterns have been reported to lack response to perturbations: minimal changes were observed after treatment with antifolates and chloroquine; however, larger changes have been observed with other drug classes [236,237]. Species-specific differences in transcription have been observed that appear to be linked to the mammalian host[238].
- Ribosome profiling demonstrated that transcription and translation are tightly coupled for 90% of genes[239]. Exceptions of translationally upregulated genes typically were found for proteins involved in merozoite egress and invasion.
- Epigenetic mechanisms to control gene expression include post-translational histone modifications (methylation and acetylation of the N-terminus are the best-characterized). Many of these modifications have been linked to parasite development.[64,240].

[H1] Genomic tools

- Gene knockouts are possible, but RNA interference -mediated knockdown mechanisms do not function in *Plasmodium* species[241,242].
- Regulated RNA aptamer-based approaches have led to methods that allow gene knockouts to be functionally rescued, a key method to study essential genes [241,242].
- CRISPR-Cas9 directed genome editing has greatly facilitated genetic manipulation of *P. falciparum*. [241,242].
- Bar coded mutant *P. berghei* libraries have been developed to screen for competitive fitness across tens of mutants in a single mouse[243].
- *In vitro* selection of drug-resistant mutant parasites followed by whole-genome sequencing has also become a well-established method to reveal candidate drug-targets[244].
- Metabolomics approaches facilitate understanding of *Plasmodium* biology and have been used to profile a number of antimalarial compounds of both known and unknown mechanisms of action[245].

Box 3: Malaria and Pregnancy

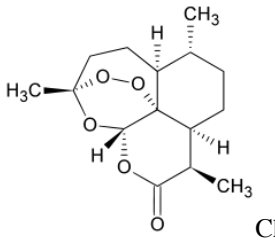
- Pregnant woman are more-susceptible to *Plasmodium* infection, particularly in the first pregnancy, as the mother-to-be has not yet acquired immunity to parasites expressing the protein VAR2CSA [36]. VAR2CSA on the surface of infected red blood cells facilitates adhesion to chondroitin sulphate A (which is expressed by placental proteoglycans), leading to sequestration in the placenta [7,65]. The risk of placental malaria is reduced in multigravida women from endemic areas, who generally have antibodies against VAR2CSA [66-68]
- Malaria during pregnancy leads to increased risks to the mother and foetus[37,246]. Most studies have focused on sub-Saharan Africa; however, pregnancy-related risks are a problem throughout the world, including Latin America, where *P. vivax* is the dominant causative agent[247]
- Placental malaria might be asymptomatic or clinically mild, but also leads to increased risk of death for both foetus and mother. It predisposes to miscarriage, stillbirth, preterm delivery and babies with low birth weight, whose quality of life will probably be poor because of cognitive, mobility, self-care and sensation limitations and a high mortality rate [37,246].
- Intermittent preventive treatment with sulfadoxine-pyrimethamine in endemic regions is recommended, and is generally administered at each antenatal visits following quickening[109], though the emergence of resistance is threatening its efficacy.[248]
- Treatments for pregnant woman must take into account the availability of safety data for the foetus. As a consequence, newer treatments require time to obtain sufficient confirmation of their tolerability in the different trimesters. The WHO recommends quinine sulphate and clindamycin in the first trimester. Artemisinin derivatives provided comparable safety to quinine [249], but the results of this study have not yet been incorporated into the WHO guidelines. In the second or third trimester, the WHO recommends artemisinin-based combination therapies[109].
- Treatment of pregnant women with *P. vivax*, *P. ovale* or *P. malariae* infection can also include chloroquine, unless resistance is suspected[109]. Women at high risk for relapses can be given weekly chloroquine chemoprophylaxis until after delivery. Follow up

therapy with primaquine against *P. vivax* and *P. ovale* hypnozoites is not thought safe in pregnancy.

Box 4: Artemisinin

Artemisinin (also known as qinghaosu in China) is extracted from the leaves of the *Artemisia annua* plant.

Youyou Tu was recognized by the 2015 Nobel Prize committee for her contribution to medicine for the discovery of artemisinin, by retrieving and following instructions from ancient Chinese texts [250]. Thanks to the ability of artemisinin to rapidly reduce parasitemia and fever, the effect that artemisinin and its derivatives had on the management of malaria cannot be overstated: since their introduction in the 1970s and subsequent wider implementation, which was possible particularly owing to the work of Prof. Nicholas White and colleagues [251-254], millions of lives were saved. These drugs appear to be activated by heme derived iron and their toxicity is probably mediated through the formation of reactive oxidative radicals[43]. Data suggest that they interfere with phosphatidylinositol-3-phosphate (PI3P) metabolism (which is thought to be involved in the trafficking of haemoglobin to the digestive vacuole[255]) and provide possible mechanistic insight into the nature of clinically observed artemisinin resistance[256].



Chemical structure of artemisinin

Figure 1: *Plasmodium* life cycle. The mosquito vector transmits the *Plasmodium* parasite in the sporozoite stage to the host during a blood meal. Sporozoites invade liver cells, where they replicate and divide as merozoites. The infected liver cell ruptures, releasing the merozoites into the blood stream, where they invade red blood cells and begin the asexual reproductive stage, which is the symptomatic stage of the disease. Symptoms develop 4-8 days after the initial red blood cell invasion. The replication cycle of the merozoites within the red blood cells lasts 36-72 hours (from red blood cell invasion to haemolysis). Thus, in synchronous infections (infections that originate from a single infectious bite), fever occurs every 36-72 hours, when the infected red blood cells lyse and release endotoxins *en masse*[71-73] . P. *vivax* and P. *ovale* can also form a dormant state in the liver, the hypnozoite. Merozoites released from red blood cells can invade other red blood cells and continue to replicate or, in some cases, they differentiate into male or female gametocytes [4,5]. The transcription factor AP2-G has been shown to regulate the commitment to gametocytogenesis. Gametocytes concentrate in skin capillaries and are then taken up by the mosquito vector in a blood meal. In the gut of the mosquito, each male gametocyte produces eight microgametes after three rounds of mitosis; the female gametocyte matures into a macrogamete. Male microgametes are motile forms with flagellae and seek the female macrogamete. Once in the mosquito male and female gametocytes fuse, forming a diploid zygote, which elongates into an ookinete, a motile form that exits from the lumen of the gut across the epithelium[257] as an oocyst. These undergo cycles of replication, and form sporozoites, which move from the abdomen of the mosquito to the salivary glands. Thus, 7-10 days after the mosquito feeds on blood containing gametocytes, it is armed and able to infect another human with *Plasmodium* with her bite. Drugs that prevent *Plasmodium* invasion or proliferation in the liver have prophylactic activity, drugs that block the red blood cell stage are required for treatment of the symptomatic phase of the disease and compounds that inhibit the

formation of gametocytes or their development in the mosquito (including drugs that kill mosquitoes) are transmission-blocking agents. The Figure is modified from [258]

*this can be delayed by months or years in case of hypnozoites

‡ until symptoms

§ differs by species

|| highly temperature dependent

Figure 2: Map of malaria endemic regions (adapted from the 2015 WHO World Malaria report)[16]The most deadly malaria parasite, *P. falciparum,* is only found in tropical areas because its gametocytes requires 10-18 days at a temperature of > 21^{o}C to mate and mature into infectious sporozoites inside the vector[259]. This development timeline is possible in hot, tropical conditions only; where the ambient temperature is lower, mosquitoes can still propagate, but sporozoite maturation is slowed down and, therefore, incomplete, and parasites perish without progeny when the mosquitoes die. Thus, *P. falciparum* is quite temperature-sensitive; a global temperature rise of 2-3° C might result in an additional 5% of the world population (that is, several hundred million people) being exposed to malaria.[260] Of note, *P. vivax* and *P*. *ovale* can develop in mosquitoes at ambient temperature as low as 16°C, The ability to propagate at sub-tropical temperatures and to remain in hypnozoite state in the liver likely explain the broader global distribution of these parasites and their ability to elude elimination during the cold season in temperate zones[261]. Countries coded 'not applicable' were not separately surveyed.

Figure 3: Parasite entry and replication within the red blood cells

Invasion occurs in a multi-step process. [262] During preinvasion, low-affinity contacts are formed with the red blood cell membrane. Reorientation of the merozoite is necessary to allow close contact between parasite ligands and host cell receptors, and this is then followed by tight junction formation. In *Plasmodium falciparum*, a forward genetic screen showed that

complement decay-accelerating factor (CD55) on the host red blood cell was essential for invasion of all *P. falciparum* strains[263].The interaction of a complex of *P. falciparum* proteins (*P. falciparum* reticulocyte-binding protein homolog 5 (*Pf*Rh5), *P. falciparum* RH5-interacting protein (*Pf*Ripr) and cysteine-rich protective antigen (CyRPA)) with basigin on the red blood cell surface is also essential for invasion in all strains[264,265]. PfRH5 has been studied as a potential vaccine candidate[47] and antibodies against basigin have been considered as a potential therapeutic strategy[266]. With the PfRh5/PfRipr/CyRPA-basigin binding step, an opening forms between the parasite and the red blood cell, which triggers $Ca2^{+}$ release and enables parasite released proteins to be inserted into the red blood cell membrane. These proteins are secreted from the micronemes (the smallest secretory organelles that cluster at the apical end of the merozoite) and the neck of the rhoptries and include Rhoptry neck protein 2 (RON2). Binding between RON2 and apical membrane antigen 1 (AMA1) proteins on the merozoite surface is required to mediate tight junction formation prior to the internalization process[267], and AMA1 is also being evaluated as a vaccine candidate [268]. Parasite replication within the red blood cell requires the synthesis of DNA, which can be blocked by several antimalarials: pyrimethamine (PYR), P218 and cycloguanil target *Plasmodium* dihydrofolate reductase (PfDHFR) [269] and atovaquone (ATO) blocks pyrimidine biosynthesis by inhibiting *Plasmodium* cytochrome b mitochondrial gene (*Pfcytb*) and preventing the formation of oxidized Coenzyme Q, which is needed for the pyrimidine biosynthetic enzyme dihydroorotate dehydrogenase (PfDHODH) to perform its reaction within the mitochondria [51]. The Phase II clinical candidate DSM265 also blocks pyrimidine biosynthesis by directly inhibiting PfDHODH[189]. Besides DNA synthesis, other processes can be targeted by antimalarial drugs.

Chloroquine (CHQ) inhibits heme polymerization in the food vacuole [53], but can be expelled from this compartment by the *Plasmodium* chloroquine-resistance transporter (PfCRQ)[270]. The Phase II clinical candidate Cipargamin and preclinical candidate SJ733 both

inhibit PfATP4, which is required for Na+ homeostasis during nutrient acquisition [58,186,187]. The Phase I clinical candidate MMV048 [194] inhibits phosphatidylinositol-4 kinase (PI(4)K), which is needed for the generation of transport vesicles that are needed to promote membrane alterations during ingression [59].

Figure 4: Microscopic images of parasite-infected red blood cells. Thin blood films showing A. *P falciparum* and B. *P. vivax* at different stages of blood stage development. ER, early ring stage; LR, late ring stage; ET, early trophozoite; LT, Late trophozoite stage; ES, early schizont stage; LS, late schizont; FM, free merozoites; U, uninfected red blood cell. Gender Symbols represent microgamete (Male symbol) and Macrogamete (Female symbol) Images (100x Oil immersion) from Methanol fixed Thin Films stained for 30 minutes in 5% Giemsa. Samples taken from Thai and Karen malaria patients: Ethical Review Committee for Research in Human Subjects, Ministry of Public Health, Thailand (reference no. 4/2549, 6 February 2006). Slides used from a previously published study[271], provided by Alice-Roza Eruera and Bruce Russell (University of Otago)

Figure 5. Global pipeline for malaria vector control. The categories of compounds currently under research are defined in the first column on the left; compounds belonging to these categories have advanced to Phase I trials or later stages. New screening hits (developed by Syngenta, Bayer and Sumitomo/IVCC) are at early research stages and not expected to be deployed until 2020-2022. Similarly, species-specific, biological control of mosquitoes approaches are not expected to move forward before 2025. Key. AI: active ingredient; IRS, indoor residual spray; IVCC: Innovative Vector Control; LLIRS, long-lasting indoor residual spray; LLITN: long-lasting insecticidal mosquito net, LLN, long-lasting net: LSHTM, London School of Hygiene and Tropical Medicine; PAMVERC, Pan-African Malaria Vector Research

Consortium; [a]clothianidin and chlorfenapyr. The main data source was the Innovative Vector Control Consortium, for the latest updates visit www.ivcc.coml; note that not all compounds listed are shown here. Dates reflect expected deployment.

Figure 6: Global pipeline for malaria vaccines.

Key. AMANET, African Malaria Network Trust; ASH, Albert Schweitzer Hospital; CHUV, Centre Hospitalier Universitaire Vaudois; CNRFP, Centre National de Recherche et de Formation sur le Paludisme; ee, elimination eradication; EVI: European Vaccine Initiative; FhCMB : Fraunhofer Center for Molecular Biotechnology, USA; GSK: Glaxo SmithKline; IP, Institut Pasteur; INSERM: Institut national de la santé et de la recherche médicale, France; JHU: Johns Hopkins University; KCMC: Kilimanjaro Christian Medical College, Tanzania; KMRI, Kenyan Medical Research Institute; LSHTM, London School of Hygiene and Tropical Medicine; LMIV, Laboratory of Malaria Immunology and Vaccinology; MRCG, Medical Research Council (The Gambia); NIAID: National Institute of Allergy and Infectious Diseases, USA; NHRC, Navrongo Health Research Centre; NIMR, National Institute for Medical Research; NMRC: Naval Medical Research Center; MUK, Makerere University Kampala ; pp, pediatric prevention; SST, Statens Serum Institut; U.: University; UCAP, Université Cheikh Anta Diop ; UKT, Institute of Tropical Medicine, University of Tübingen; USAMMRC: US Army Medical Research and Materiel Command; WEHI: Walter and Eliza Hall Inst. of Medical Research; WRAIR, Walter Reed Army Institute of Research. Main source: WHO 'Rainbow Tables'[272] Not all vaccines under development are listed here.*Pending review or approval by WHO pre-qualification, or by regulatory bodies who are ICH members or observers; [‡]Sponsors for late-stage clinical trials.

Figure 7: Global pipeline for antimalarial drugs showing current product profiles. A. Preclinical candidates. B. Compounds or compound combinations in clinical Development. The multitude of molecules targeting only asexual blood stages reflects the fact that many of these compounds are at an early stage of development, and further assessment of their target candidate profile is still on going. KAF156 and KAE609 were discovered in a multi-party collaboration between Novartis Institute for Tropical Disease, Genomics Institute of the Novartis Research Foundation, Swiss Tropical & Public Health Institute, Biomedical Primate Research Centre, Wellcome Trust and MMV. DSM was discovered by a collaboration involving University of Texas Southwestern, University of Washington, Monash University, GSK and MMV. MMV048 was discovered through a collaboration involving University of Cape Town, Swiss Tropical and Public Health Institute, Monash University, Syngene and MMV. SJ733 was discovered in a collaboration involving St Jude Children's Research Hospital, Rutgers University, Monash University and MMV. Note that not all compounds are listed here and updates can be found at www.mmv.org.

[a]3-day cure, artemisinin-based combination therapy

[b]Part of a combination aiming at a new single-exposure radical cure (TPP-1)

[c]Severe malaria and pre-referral treatment

[d]Product targeting prevention of relapse for *P. vivax*

Figure 8. Chemical structures of novel non-artemisinin based compounds in clinical development.

Key. MMV, Medicines for malaria venture; GSK: Glaxo SmithKline; CDRI, Central Drug Research Institute; UCT, University of Cape Town

[a]3-day cure, artemisinin-based combination therapy

[b]Part of a combination aiming at a new single-exposure radical cure (TPP-1)

[c]Product targeting prevention of relapse for *P. vivax*

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Table 1: The artemisinin-based combination therapies within the portfolio of Medicines for Malaria Venture*

Drug combination	Oral formulation (adults, children)	Number of patients treated (million)	Number of countries where approved	Brand name (manufacturer)	Regulatory body (approval date)
Artesunate Amodiaquine Winthrop	Oral formulation , dispersible	>400	33	ASAQ Winthrop (Sanofi, DNDi and MMV)	WHO (2008)
Artemether-Lumefantrine	Oral formulation , dispersible	>300	>50	Coartem D® (Novartis and MMV)	Swiss Medic (2008), FDA (2012)
DHA-Piperaquine	Coated tablets, dispersible‡	2	11	Eurartesim®, (Sigma Tau and MMV)	EMA (2011); Prequalification (2015)
Artesunate-Pyronaridine	Oral formulation, granules	Pending inclusion on Standard treatment guidelines	20	Pyramax®, (Shin Poong and MMV)	EMA Article 58 and WHO Prequalification (2012) then positive opinion(2015) for granules and multiple use
Artesunate-Mefloquine	granules	N/A	10	No brand name (Farmanguinhos, Fiocruz, DND*i*, Cipla and MMV)	Cipla WHO Prequalified (2012) Farmanguinhos Pending

*In general, artemisinin-based combination therapies target all *Plasmodium* species.

‡paediatric formulation to be submitted

MMV; www.mmv.org. FDA: (US) Food and Drug Administration; DHA, dihydroartemisinin; DND*i* : Drugs for Neglected Diseases *initiative*; EMA: European Medicines Agency. N/A, not available

Table 2. Drug resistance markers to clinically approved anti-malarial agents.

Drug	***P. falciparum* Resistance Marker (gene, protein; PlasmoDB gene ID)**	**Protein function**	**Geography and resistance reports**
Artemisinin derivatives	*K13*, Kelch protein K13; PF3D7_1343700	Scaffold protein may be involved in maintaining PI3P (phosphatidylinositol-3-phosphate) levels[256]	Greater Mekong subregion [46,273-276]
Lumefantrine	*Mdr1*, multidrug resistance protein 1; PF3D7_0523000	ATP dependent drug efflux pump from the ABC transporter B family [270,277,278]	Reports of polymorphisms Uganda, Tanzania, but no robust evidence of resistance [279-281]
Amodiaquine	*Crt*, chloroquine-resistance transporter and *Mdr1;* Pf3D7_0709000 and PF3D7_0523000	drug metabolite/transporter superfamily of electrochemical potential-driven transporters[282]	Africa, Asia[280,283]
Mefloquine	*Mdr1;* PF3D7_0523000	drug metabolite/transporter superfamily of	Greater Mekong subregion[284-286]

		electrochemical potential-driven transporters[282]	
Piperaquine	*HAP*; Plasmepsins II and III; *exo*, putative exonuclease gene PF3D7_1408000, PF3D7_1408100 and and PF3D7_1362500	Food vacuole histo-aspartic proteases[287]; putative exonuclease gene [171,276]	Greater Mekong subregion [171,276,288]
Pyronaridine	None reported	N/A	No robust reports

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Figure 4: permission lines needed

Subject categories:

Health sciences / Diseases / Infectious diseases / Malaria

[URI /692/699/255/1629]

Biological sciences / Immunology / Infectious diseases / Malaria

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Health sciences / Health care / Public health

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ToC blurb

Malaria is a mosquito-transmitted infection that affects over 200 million people worldwide, with the highest morbidity and mortality in Africa. Eradication, through vector-control approaches and chemoprevention, is within reach, but threatened by the emergence of drug-resistant strains of mosquitoes and *Plasmodium*, the infectious parasite