Malaria: biology and disease

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Summary

Malaria has been a major global health problem of humans through history and is a leading cause of death and disease across many tropical and subtropical countries. Over the last fifteen years renewed efforts at control have reduced the prevalence of malaria by over half raising the prospect that elimination and perhaps eradication may in the long term be possible. To achieve this goal will require the development of new tools including novel antimalarial drugs and more efficacious vaccines as well as an increased understanding of the disease and the biology of the malaria parasite. This has catalysed a major effort that has resulted in the development and regulatory approval of the first vaccine against malaria (RTS,S/AS01) as well as the identification of novel drug targets and antimalarial compounds some of which are in human clinical trials.

Introduction

Malaria remains a disease of global public health importance with 3.3 billion people in 97 countries at risk, leading to an estimated 200 million cases and around 600,000 deaths (WHO, 2015). Five plasmodial species present a significant health threat for humans; *Plasmodium falciparum* is usually considered the most important in terms of deaths, and this review will focus largely on this parasite. *Plasmodium vivax* is a major cause of illness across large parts of the world and it is increasingly argued that deaths due to this parasite have been underestimated (Baird, 2013; Naing et al., 2014). *P. ovale* and *P. malariae* are much less common causes of significant disease. Recently the simian parasite *P. knowlesi* has emerged as a local but important cause of disease (including severe disease) in Malaysia and other areas of south east Asia (Ahmed and Cox-Singh, 2015), where it currently seems to be predominantly a zoonosis, with no definite evidence of primary human to human transmission.

Historically *P. falciparum* has probably exerted greater selective pressure on human evolution than any other single pathogen. Although present throughout tropics, the health impact is far from even. The large majority of the world's parasitised individuals live in Asia and south Asia, reflecting the large human population but 90% of deaths occur in Africa, mostly in children (WHO, 2015). Over recent years there have been renewed calls for elimination and eventual eradication of malaria. In this review, we will outline the major features and recent developments in understanding the biology, epidemiology and clinical consequences of malaria, which will be critical in developing new approaches to prevention and treatment necessary to tip the balance from control to elimination.

Biology of malaria

Plasmodium spp. are global pathogens that have a complex life cycle alternating between the female *Anopheles* mosquito and vertebrate host and this requires the ability of unique zoite forms to invade different cell types at specific stages (Figure 1). Once the sporozoite form enters the host it infects hepatocytes followed by the asexual cycle in the blood. Sexual forms that develop during the blood stage are passed to a biting mosquito during feeding to complete the cycle. There is some understanding of the molecular events for the infection, survival and development of each of these stages. However, in this review we will concentrate on the stages within the host and in particular the liver and blood stages.

Infection of the liver

The sporozoite form of *Plasmodium spp.* is injected into the dermis of the host during a blood feed (Figure 1). The fate of these sporozoites is not well understand and most of the information has come from study of the mouse malaria model *P. berghei* where it has been observed that they can take 1 – 3 hours to exit from this site (Amino et al., 2008; Amino et al., 2006). In the dermis they display gliding motility, which is a random process that enables a proportion to penetrate a blood vessel and enter the bloodstream. Those that remain in the skin can be destroyed although some are intracellular but many are drained by the lymphatics where a host immune response can be generated. The protein named Trap-like protein (TLP), which has von Willebrand factor A (vWA) and thrombospondin type 1 (TSP1)domains, may play a role in exit from the dermis as mutant sporozoites lacking this protein display normal gliding motility (Hellmann et al., 2011; Moreira et al., 2008). Those that enter the blood stream quickly access the liver by crossing the sinusoidal barrier composed of fenestrated endothelial and Kupffer cells, which are resident macrophages and this may or may not be associated with traversal through these cell types (Tavares et al., 2013). A number of proteins required for traversal have been identified including SPECT (sporozoite microneme protein essential for traversal) (Ishino et al., 2004), SPECT2 (also known as perforin-like protein 1, PLP1) (Ishino et al., 2005; Kaiser et al., 2004; Risco-Castillo et al., 2015), CelTOS (Cell traversal protein for ookinetes and sporozoites) (Kariu et al., 2006), phospholipase (PL) (Bhanot et al., 2005) and gamete egress and sporozoite traversal protein (GEST) (Talman et al., 2011). The function of these proteins in cell traversal is not understood although SPECT2 has a membrane attack complex/perforin-like domain suggesting it plays a role in punching holes in membranes. Sporozoites traverse through cells by forming a transient vacuole and SPECT2 and pH sensing appears to be involved in egress from this structure (Risco-Castillo et al., 2015).

It has been suggested that the process of cell traversal through the sinusoidal barrier is important for infectivity (Baer et al., 2007; Ishino et al., 2005; Ishino et al., 2004; Kariu et al., 2006) by priming the sporozoite for invasion of hepatocytes, the cell in which they divide and expand (Mota et al., 2002). However, more recent studies have shown the primary role of cell traversal inhibits sporozoite clearance by Kupffer cells during movement of the sporozoite inside the sinusoid lumen, before crossing the barrier (Tavares et al., 2013). Sporozoites injected into the dermis are in a state called 'migratory mode' and they convert to an 'invasive mode', most likely through multiple signals, once they interact with hepatocytes. One such signal involves the recognition of hepatocytes through binding higher sulfated forms of heparin sulfate proteoglycans (HSPGs) and this activates the calcium dependent protein kinase 6 (CDPK6), which is linked to the sporozoite switch to an invasive form (Coppi et al., 2007). Additionally, the tetraspanin CD81 and scavenger receptor B1 (SR-B1) are required for invasion of human hepatocytes by *P. falciparum* and formation of a parasitophorous vacuole (Rodrigues et al., 2008; Silvie et al., 2003; Yalaoui et al., 2008) In contrast, the hepatocyte receptor EphA2 is not required for hepatocyte invasion but plays an essential function in establishment of the parasitophorous vacuole through interaction with the parasite 6-cys proteins p52 and p36 (Kaushansky et al., 2015).

The sporozoite is covered by a dense coat and one of the key proteins is the circumsporozoite protein (CSP) and it consists of a highly repetitive region and a type I thrombospondin repeat (TSR) at the C-terminus (Ozaki et al., 1983). Invasion of the hepatocyte by the sporozoite requires binding of CSP to highly sulfated HSPGs activating processing of CSP and removal of the N-terminus thus exposing the TSR domain (Coppi et al., 2011; Herrera et al., 2015; Kojin et al., 2016). Subsequent steps involve proteins with adhesive domains that have been released from the apical organelles (micronemes and rhoptries) a process that is activated by mobilization of Ca^{2+} within the sporozoite. This includes two micronemal type 1 membrane proteins thrombospondin related anonymous protein (TRAP) (Robson et al., 1995; Robson et al., 1988) and apical membrane antigen-1 (AMA-1) (Silvie et al., 2004). TRAP binds to highly sulfated HSPGs and has tandem von Willebrand factor A (VWA) and TSR repeat domains and there is evidence suggesting the cytoplasmic domain connects directly to the actomyosin motor in the parasite through aldolase that provides the force for gliding motion and invasion (Bosch et al., 2007). However, the demonstration in the related apicomplexan parasite *Toxoplasma gondii* that aldolase is dispensable for tachyzoite invasion renders this model unlikely and currently it is not clear how the adhesive proteins involved in parasite movement are linked to the actomyosin motor (Shen and Sibley, 2014). TRAP appears to be

important for sporozoite invasion by binding to HSPGs, however, beyond that how it functions in this process is unclear.

In contrast, AMA-1 has been implicated in formation of the tight attachment, that has been called the tight junction, that links the invading parasite and host cell to the parasite actomyosin motor (Silvie et al., 2004). This tight junction moves along the parasite surface as it invades the host cell and in *T. gondii* it has been shown that as the tachyzoite apical end forms a tight attachment to the host cell a RhOptry Neck protein (RON) complex is injected under and into the host cell membrane and RON2 forms a complex with AMA-1 (Besteiro et al., 2011; Besteiro et al., 2009). This provides the physical link between the host cell and parasite membrane and ultimately the actomyosin motor. The role of AMA-1 in tight junction formation has been questioned by the demonstration that AMA-1 in *P. berghei* is not absolutely required for sporozoite invasion as the gene can be conditionally deleted (Bargieri et al., 2013; Giovannini et al., 2011). However, RON2 is essential for/in tight junction formation and this suggested that it must bind to proteins other than AMA-1 to form this structure and in *T. gondii* this has been shown to be AMA-1-like proteins (AMA2) (Lamarque et al., 2014; Poukchanski et al., 2013). This is likely similar across *Plasmodium spp.* as there are other AMA1-like proteins that play a role in sporozoite invasion such as MAEBL (Saenz et al., 2008; Steinbuechel and Matuschewski, 2009). So it appears that the sporozoite provides both ligand and receptor to form the tight junction thus physically linking the host cell membrane and parasite membrane and then in some way to the actomyosin motor that provides the force to pull the parasite into the host. This is a conserved process operating in *Plasmodium spp.* and other apicomplexan parasites as well as other stages of the *P. falciparum* and *P. vivax* life cycle (see below).

Egress of merozoites and invasion of red blood cells

Once the sporozoite invades the hepatocyte it develops into thousands of hepatic merozoites that are released into the blood stream through budding of parasite-filled vesicles called merosomes (Sturm et al., 2006) (Figure 1). The free merozoites quickly invade red blood cells and this dynamic process involves multiple steps that have been visualised by video-microscopy for *P. falciparum* that includes pre-invasion, active invasion and echinocytosis (a process in which the red blood cell rapidly shrinks forming spiky protrusions) (Gilson and Crabb, 2009) (Figure 2). Pre-invasion involves the interaction of the merozoite with the red blood cell and surprisingly little is understood about the molecular detail of this step. The merozoite surface protein 1 (MSP1) is the major glycophosphatidylinositol (GPI) on the

merozoite surface (Gilson et al., 2006; Holder, 1994) that undergoes proteolytic processing by the protease SUB1 (Silmon de Monerri et al., 2011) and it has been implicated in direct binding to band 3 (Baldwin et al., 2015; Goel et al., 2003) on the red blood cell surface during pre-invasion. MSP1 acts as a platform on the merozoite surface for formation of at least three large molecular weight complexes that involves different extrinsic proteins that are able to bind red blood cells (Lin et al., 2016; Lin et al., 2014). However, MSP1 has been shown to be required for egress of merozoites from the *P. falciparum* infected red blood cell through SUB1 processing on the merozoite surface that activates its ability to bind spectrin (Das et al., 2015). Additionally, merozoites lacking MSP1 are able to invade red blood cells suggesting it is not absolutely required for invasion. It is possible that MSP1 is involved in display of proteins involved in evasion of host responses rather than directly in merozoite invasion. Indeed, it has been recently shown that the GPI-anchored merozoite surface protein Pf92 (a member of the 6-cys family) acts to bind and recruit the complement regulatory factor H to the merozoite surface thus providing an evasion strategy against complement-mediated lysis (Kennedy et al., 2016) and the MSP1 complexes could potentially play similar roles

The pre-invasion step involves a robust interaction of the merozoite with the red blood cell resulting in deformation of the host cell that requires the parasite actomyosin motor (Weiss et al., 2015) (Figure 2). This step in invasion involves two ligand families of type 1 membrane proteins that were first identified in *P. vivax* and *P. knowlesi* (Adams et al., 1992; Galinski et al., 1992; Haynes et al., 1988). These ligands bind specific receptors that include the Duffy-binding protein (PvDBP) (Adams et al., 1992; Haynes et al., 1988) and the reticulocyte-binding protein (PvRBP) families (Galinski et al., 1992). The equivalent protein families in *P. falciparum* are erythrocyte binding-like proteins (EBLs) and *P. falciparum* reticulocyte-binding protein homologues (PfRhs) (Rayner et al., 2000; Triglia et al., 2001). These protein ligands also bind to specific receptors and those so far identified include glycophorin A, B, C (Maier et al., 2003; Mayer et al., 2009; Mayer et al., 2006; Orlandi et al., 1992) and complement receptor 1 (CR1) (Tham et al., 2010) and whilst the function of individual members of these families appear to display redundancy the overall function of these families is essential in *P. falciparum* (Lopaticki et al., 2011). Additionally, CD55 has been identified as an essential host receptor for invasion of *P. falciparum* merozoites, however, the parasite ligand is at this stage unknown (Egan et al., 2015). Whilst the PfRh and EBL proteins are involved in sensing erythrocytes they also appear to play a role in signalling for activation of subsequent steps in the invasion process. It has been suggested that exposure of merozoites to the low potassium ion concentrations in blood plasma, when they egress from the host cell, leads to a rise in

cytosolic calcium levels through a phospholipase C mediated pathway and this triggers release of EBA-175, a member of the EBL family in *P. falciparum* (Singh et al., 2010). Binding of EBA-175 to its receptor, glycophorin A, triggers release of proteins from the rhoptries. This work provides evidence of the importance of these protein families in sensing and binding to erythrocytes but also signaling downstream invasion events. Indeed, PfRh1 has been shown to be linked to Ca^{2+} signalling in the merozoite (Gao et al., 2013) and phosphorylation of the cytoplasmic tail of PfRh4 by the *P. falciparum* casein kinase 2 (PfCK2) is required for invasion through the PfRh4-CR1 interaction parasite-host interaction (Tham et al., 2015). Also calcineurin plays an important role in attachment of the meroztes most likely through stabilizing the dimerization of EBL and PfRh proteins as this is critical for their ability to bind to host receptor and signal to the merozoite for subsequent events in invasion (Paul et al., 2015).

Following deformation of the red blood cell the merozoite appears to reorientate so that the apical end abuts the erythrocyte membrane. This step involves the function of PfRh5, which is a member of the PfRh family (Baum et al., 2009; Hayton et al., 2008), however, is not a type 1 membrane protein and it forms a complex with PfRipr (Rh5-interacting protein) (Chen et al., 2011) and CyRPA (cysteine-rich protective antigen) (Dreyer et al., 2012; Reddy et al., 2015). PfRh5 binds to the host receptor basigin and it plays an essential role in merozoite invasion (Crosnier et al., 2011). The PfRh5 complex-basigin interaction is associated with a Ca^{2+} spike that occurs between the merozoite and red blood cell that moves into the host cell (Volz et al., 2016; Weiss et al., 2015). This data suggests that there is a discontinuity or pore formed at this step through which material can pass into the red blood cell. Indeed, it is known that the merozoite injects proteins into and under the red cell membrane analogous to that occurring for sporozoite invasion of hepatocytes and it has been hypothesised that PfRh5 plays a role in this process (Weiss et al., 2015). It has also been hypothesised that Ca^{2+} is injected into the red blood cell at this point from the rhoptries, however, its source is not well established and it may be leaking from the medium through the discontinuity formed between the parasite and red blood cell.

Irreversible attachment of the merozoite to the erythrocyte occurs by formation of a tight junction, composed primarily of *P. falciparum* apical membrane antigen (PfAMA1) and the RON complex (Alexander et al., 2005). The RON complex is deposited in the erythrocyte with RON2 spanning the host membrane and binding to PfAMA1 on the merozoite surface (Alexander et al., 2005; Besteiro et al., 2011; Besteiro et al., 2009; Richard et al., 2010). The lipid rich rhoptry contents are released from the bulb region to form the parasitophorous vacuole membrane as the merozoite is propelled into the erythrocyte using force generated by

the parasite acto-myosin motor (Riglar et al., 2011). After the active invasion phase, echinocytosis occurs causing the erythrocyte to shrink forming spiky protrusions, likely caused by entry of Ca^{2+} into the erythrocyte (Gilson and Crabb, 2009; Weiss et al., 2015). After a period of echinocytosis, the infected erythrocyte recovers its biconcave shape. Overall, the invasion process is rapid taking less than two minutes (Gilson and Crabb, 2009; Weiss et al., 2015).

Active invasion involves irreversible attachment of the merozoite to the erythrocyte by formation of a tight junction and this appears to be composed primarily of *P. falciparum* apical membrane antigen (PfAMA1) and the RON complex (Alexander et al., 2005; Besteiro et al., 2011; Besteiro et al., 2009; Richard et al., 2010). The RON complex is deposited in the erythrocyte with RON2 spanning the host membrane and binding to PfAMA1 on the merozoite surface. The lipid rich rhoptry contents are released from the bulb region to form the parasitophorous vacuole membrane as the merozoite is propelled into the erythrocyte using force generated by the parasite actomyosin motor (Riglar et al., 2011). Fusion of membranes at the posterior end of the merozoite occurs to seal the parasite within the parasitophorous vacuole and the erythrocyte. There is some evidence to suggest that AMA1 is involved in this resealing process but the mechanism is unknown (Yap et al., 2014). After the active invasion phase, echinocytosis occurs causing the erythrocyte to shrink forming spiky protrusions and it has been suggested that this is caused by entry of Ca^{2+} into the erythrocyte during interaction of the PfRh5 complex with basigin (Gilson and Crabb, 2009; Weiss et al., 2015). After a period of echinocytosis, the infected erythrocyte recovers its biconcave shape. Overall, the invasion process is rapid taking less than two minutes (Gilson and Crabb, 2009; Weiss et al., 2015).

Once the merozoite has invaded it begins to grow and eventually undergoes division resulting in 16-32 merozoites that when fully develop egress resulting in destruction of the red blood cell membrane and an explosive release of the parasites allowing them to access new host cells for invasion (Glushakova et al., 2005; Wickham et al., 2003). The coordinated process of merozoite egress is tightly regulated and involves a number of protein kinases including the plant-like calcium-dependent protein kinase PfCDPK5 (Dvorin et al., 2010) and cGMP-dependent protein kinase (PfPKG) is essential for egress (Collins et al., 2013). Additionally, as mentioned previously MSP1 appears to play an essential role in egress through interaction with spectrin although its exact mechanism is not yet known (Das et al., 2015).

Remodeling of the host cell and immune evasion

Following invasion of merozoites into the red blood cell the parasite activates a remarkable process of renovation that turns a highly specialized terminally differentiated cell, lacking most of the basic machinery and organelles, into one in which the parasite can grow and hide from host responses (reviewed in (Boddey and Cowman, 2013)). It does this by exporting hundreds of proteins out of the parasite and beyond the parasitophorous vacuole membrane to numerous locations within the parasite-infected erythrocyte (Hiller et al., 2004; Marti et al., 2004; Sargeant et al., 2006) (Figure 3). This is dependent on the construction by the *P. falciparum* parasite of a trafficking network in the infected host cell responsible for sorting and moving these exported proteins to specific subcellular locations. A key feature of this network are Maurer's clefts which are membranous structures in the shape of flattened disc that bud from the parasitophorous vacuole soon after invasion (Gruring et al., 2012; McMillan et al., 2013). These structures slowly move to the underside if the infected erythrocyte where they become tethered to the underside of the red cell membrane at about 20 hours post invasion. A small transmembrane protein MAHRP2 (membrane-associated histidine-rich protein-2) has been shown to be a component of the Maurer's cleft tether (Pachlatko et al., 2010). There are other smaller membranous structures that have been observed in the *P. falciparum*-infected erythrocyte that have been called EDV (electron dense vesicles) and J-dots, however, their function is unknown although they have been implicated in trafficking of proteins from Maurer's clefts to the host cell membrane (Gruring et al., 2012; Kulzer et al., 2010; Spycher et al., 2006).

An important protein that is exported to the surface of the *P. falciparum*-infected erythrocyte is the *P. falciparum* erythrocyte membrane protein 1 (PfEMP1), a family of proteins that shows enormous diversity and is responsible for cytoadherence and antigenic variation (Baruch et al., 1995; Smith et al., 1995; Xin-zhuan et al., 1995) (Figure 3). The role of this protein family in malarial immunity and pathogenesis has recently been reviewed (Hviid and Jensen, 2015). The PfEMP1 family is encoded by approximately 60 genes in each *P. falciparum* parasite with monoallelic transcription so that only one form of this protein is expressed at any one time by a process regulated by epigenetic mechanisms (Duraisingh et al., 2005; Freitas-Junior et al., 2005; Gardner et al., 2002; Scherf et al., 1998). However, the expression of this gene family undergoes switching to alternative genes and this provides a mechanism of antigenic variation that is important for evasion of host immune responses (Bull et al., 1998). The different PfEMP1 proteins have distinct receptor binding selectivity and this contributes to the spectrum of disease severity and pathology of malaria (Naka et al., 2014; Nunes-Silva et al., 2015; Ochola et al., 2011). Sequestration of *P. falciparum*-infected

erythrocytes in the vasculature of different organs including the brain and placenta contributes to the severity of disease and most likely the final outcome.

The epidemiology of malaria

Malaria is transmitted in 97 countries in the world. The limits of transmission are set by environmental effects (primarily temperature) on the ability of the mosquito vector to sustain the developmental cycle of the parasite. Within these limits, the amount of transmission is determined by the frequency of contact between infected mosquitos and humans which is in turn affected by the density of vectors, their location and their feeding habits.

The level of transmission is critical in determining the clinical picture of disease due to malaria in exposed communities. In areas where the risk of infectious bites is low and unpredictable malaria is said to be unstable. Under such circumstances all ages are susceptible to infection and in general all infective episodes will result in clinical disease. As exposure rises to higher levels it reaches a point where transmission is more consistent year to year and exposed individuals begin to develop a degree of immunity.

The relationship between transmission and disease burden

Transmission intensity may range over five log orders from less than one infectious bite a year to many hundreds per day. The intensity of transmission is often expressed in terms of the proportion of a population of defined age with parasitaemia (usually asymptomatic) at a given time. In stably endemic areas severe disease and death is restricted mainly to young children, older subjects developing a degree of protective immunity (Figure 4). The higher the transmission the more quickly is immunity acquired and this affects the clinical spectrum of disease, with severe anaemia dominating in areas of very high transmission, whereas cerebral malaria becomes more dominant under moderate to low transmission settings. The relationship between severe malaria and transmission is non-linear (Snow et al., 1997) with the implication that control measures in a high transmission setting may lead to marked reductions in transmission with less effect on disease (and by implication deaths) until transmission falls to a critical point, following which reductions in disease may be rapid. This picture is supported by ongoing experience across a number of areas in Africa. Even more important may be the relationship between malaria transmission and all-cause mortality. Long standing suggestions that exposure to malaria leads to a disproportionately high number of deaths due to other causes (indirect mortality) have recently been given strong support from both epidemiologic studies (Scott et al., 2011) and from the results of direct interventions (Kleinschmidt et al., 2009).

Naturally acquired immunity to malaria

The typical picture of immunity in a stably endemic population is shown in figure 5. The risk of severe disease and death is highest in young children and falls off rapidly. Susceptibility to clinical episodes of malaria which are not life threatening continues but declines steadily such that by early adulthood febrile episodes are few and mild. Risk of infection continues throughout life. This picture is sometimes interpreted as showing that acquisition of immunity to disease ("anti disease immunity") precedes acquisition of immunity to parasite replication ("anti-parasite immunity") but this obscures a key aspect of malaria biology: even in a single episode in a naïve subject who survives the initial wave of parasitaemia, a balance is quickly struck whereby long periods are spent with low level asymptomatic parasitaemia. Thus, even at the time when children are most susceptible to severe disease and death, their *normal* state is to be asymptomatically parasitised. This implies that at this stage immunity is acquired to each parasite isolate that is survived ("strain specific immunity"), giving rise to the idea that over time increasing immunity is due to either the acquisition of a repertoire of responses to many different isolates or to the development of cross protective response to shared antigenic targets.

Although antigenic polymorphism is likely to be part of the reason for the relative slowness of acquisition of protective immunity, there is also considerable evidence that malaria infection modulates the host immune response through a number of mechanisms (reviewed in (Hviid and Jensen, 2015)). It is striking that many young children have remarkably short lived antibody responses to a number of antigens and that these often fail to boost on re exposure. The reasons for this are not understood as B cell memory in young children seems to be established and to persist over long periods, even in the absence of continuing infection (Ndungu et al., 2012).

Potential mechanisms of acquired immunity to malaria

Immune responses could potentially be targeted at any stage of the parasite to which humans are exposed. Although highly protective humoral and cellular immune responses can be developed against both the sporozoite and the intrahepatic schizont in a number of animal model systems and in humans by vaccination approaches (Riley and Stewart, 2013), there is little evidence that pre-erythrocytic responses are important in naturally acquired immunity in humans. Similarly, although humans in endemic areas make a range of antibody response to sexual stages, and experimental transmission blocking vaccine approaches against these stages

are promising (Wu et al., 2015), it is not clear what effect, if any, naturally occurring responses have on transmission.

A large body of evidence, beginning with the direct ability of gamma globulins from immune adults to rapidly control and reduce parasitaemia in children (Cohen et al., 1961) supports the idea that naturally acquired immunity is predominantly against blood stages and is antibody mediated. Antibodies could potentially act at two points: against the free merozoite before it invades red cells or against the infected red cell itself. There is clear evidence that responses to both may produce effective protection (Langhorne et al., 2008). In the case of the merozoite such responses could block the ability of the merozite to enter the red cell at several points in this process, or lead to cell mediated destruction of merozoites through a number of mechanisms including ospsonophagocytosis, ADCI or neutrophil mediated killing. Although its location within the host red cell would seem to offer protection from immune attack, the parasite remodels the host cell surface extensively, exposing an array of altered host cell and parasite antigens. Antibodies against the infected red cell surface could potentially protect by a number of mechanisms (reviewed in (Chan et al., 2014)) including cell mediated ingestion or killing, or through blocking the adhesion of the iRBC to endothelial surfaces, making it susceptible to removal in the spleen. Responses to the infected cell surface have been shown to protect from clinical disease, including severe malaria (Bull et al., 1998).

Identifying the Antigenic targets for these protective responses has been challenging. For targets on the infected red cell surface most of the evidence points to the response to PfEMP 1 being most important (Chan et al., 2012) but a significant effect of responses to other multigene families cannot be excluded. So far as the merozoite is concerned, for many years only a handful of cloned merozoite antigens were available for immune-epidemiological studies, and results were inconsistent between studies (Fowkes et al., 2010). Following the sequencing of the falciparum genome several groups have studied much larger numbers of merozoite antigens and identified a number of promising candidates (Crompton et al., 2010; Osier et al., 2014). Applying the concept of breadth and depth (ie. combinations of antibody responses to several antigens above a concentration threshold) combinations of responses which appear to give very high levels of protection have recently been identified (Osier et al., 2014)).

Clinical features of disease and pathogenesis

Malaria infection in a naïve individual almost invariably produces a febrile illness. The accompanying symptoms are non-specific and often include rigors, headache, nausea, and

muscle pains. If treated with appropriate drugs at this stage the symptoms remit over a few days, though often with considerable exhaustion. In the case of *P. falciparum*, complete treatment will eradicate the infection and any return of symptoms reflects incomplete treatment, resistance to the drugs used or new infection. In the case of *P. vivax* and *P. ovale*, subsequent infections may reoccur at intervals as a result of reactivation of the dormant hypnozoite stage, unless this is cleared by a prolonged treatment with an 8 aminoquinolone drug. The natural history of untreated or partially treated infection is well known from many thousands of observations carried out during the period when induced infections with both *P. falciparum* and *P. vivax* were used to treat neurosyphilis (Collins and Jeffery, 1999). In general, after a period of symptoms of varying severity, the illness subsides and parasite densities are controlled at a low level, only for symptoms to recur at intervals over the ensuing weeks and months, associated with rises in parasitaemia. Each successive wave of parasitaemia tends to be lower and symptoms less marked until eventually the infection resolves.

Severe malaria

In a proportion of untreated or partially treated individuals the initial infection is not controlled and progresses to severe or complicated malaria, which may lead to death. The picture of severe malaria varies with both age and the level of transmission (reflecting the immune status of populations). In Africa the large majority of malaria deaths occur in children where the picture is dominated by three syndromes which can occur separately or in combination: severe anaemia, cerebral malaria, and respiratory distress (Marsh et al., 1995). Cerebral malaria is functionally defined as the presence of coma caused by *P. falciparum* and this clinical picture may have a number of causes and is not necessarily synonymous with the histopathological description of cerebral malaria in which there is extensive sequestration of mature infected parasites in the cerebral micro vasculature (Taylor and Molyneux, 2015). Respiratory distress in children with severe malaria reflects metabolic acidosis largely reflecting tissue hypoxia (White et al., 2014a). Although acute malaria infection is associated with destruction of both infected and uninfected red cells, severe malarial anaemia in young children probably usually reflects a final "tipping over" on a background of chronic anaemia in which both immune mechanisms and ineffective erythropoiesis play a role.

In stably endemic areas of Africa severe malaria in older children and adults is very rare because of naturally acquired immunity and so our understanding of the syndrome in older subjects is based on studies in areas of the world with lower transmission, particularly in Asia. Here, although cerebral malaria, hypogalycaemia and anaemia form part of the picture, severe

malaria is more commonly a multi-system disorder often with marked renal and hepatic dysfunction (which are rare in children) (White et al., 2014a). Respiratory distress is often due to pulmonary oedema, which rarely occurs in children but has a very high mortality in adults. As previously highly endemic areas of the world experience declines in transmission (and therefore of population immunity), the mean age of severe disease and death is rising and the pattern of severe disease changing to that seen in older patients in other part of the world.

Pathogenesis of severe falciparum malaria.

The fundamental features of *P. falciparum* infection that contribute to the pathogenesis of severe disease and lead to death are exponential parasite growth, the induction of inflammatory responses in the host and microvascular obstruction caused by the adherence of mature parasites to endothelial linings of blood vessels and endothelial activation (Wassmer et al., 2015). Exponential parasite growth with greater than ten-fold increases every 48 hours means that high total body parasitaemia is achieved quickly. The importance of this may be masked by the phenomenon of sequestration of large parts of the parasite biomass adhering to the linings of blood vessels, which means that parasite load measured by microscopy is often an underestimate. Estimates of biomass based on parasite products such as HRP provide a much clearer picture (Hendriksen et al., 2013).

The exponential phase of intravascular parasite growth triggers an acute inflammatory response and it has been argued that severe malaria arises as a side effect of an excessive or poorly controlled response which is primarily evolved to control the acute infection (Cunnington et al., 2013). Systemic levels of a number of pro inflammatory cytokines have been correlated with increasing severity and death in a number of studies (Kwiatkowski et al., 1990), and IL-1β levels at birth have been reported to be predictive of both future IL-1β levels and the risk of severe malaria in childhood (Kabyemela et al., 2013). A marked pro inflammatory response is likely to contribute to the pathogenesis of severe disease in numerous ways including both systemic and local effects (Cunnington et al., 2013).

Micro vascular obstruction

A central feature of severe malaria due to *P. falciparum* is impairment of tissue blood flow associated with the sequestration of mature parasites in micro vascular beds. This contributes to both systemic effects such as metabolic acidosis and local end organ damage, most dramatically in the brain. Sequestration and subsequent impaired flow has been directly observed and quantified in a number of tissue beds including the rectal mucosa and retina and

correlates closely with clinical outcome, histological features in subjects who die and with other prognostic markers such as acidosis (Dondorp et al., 2008).

Sequestration is mediated by modifications of the red cell surface by the insertion into the IRBC membrane of variant proteins able to bind to receptors on a range of other cells (reviewed in (Smith et al., 2013)). This includes not only a large number of receptors on endothelial cells (leading to attachment within vessels) but also attachment to non-infected red cells (a phenomenon known as rosetting) and to activated platelets which leads to the formation of clumps of infected cells. Both rosetting and clumping are likely to further contribute to microvascular obstruction and have been associated with severity of disease.

Endothelial activation

It is increasingly clear that endothelial activation plays a major role in the microvascular pathology of *P. falciparum*. Nitric Oxide bioavailability is reduced (Yeo et al., 2014), and levels of Angiopoietin 2 raised (Hanson et al., 2015), and these correlate with outcome. Endothelial activation increases the expression of a number of receptors to which iRBC can bind. Whilst systemic effects from a number of cytokines may be important in inducing endothelial changes, the interaction of iRBC with the endothelium may itself lead to local endothelial activation and thus induce a vicious circle. Recently the identification of Endothelial Protein Receptor C (EPRC) as a ligand for PfEMP1 mediated iRBC binding has provided a potential explanation for how cytoadherence may lead to local endothelial dysfunction and why the brain may be particularly prone to the consequences of sequestration. EPCR is expressed in most vascular beds and plays a central role in endothelial stabilisation by promoting protein C activation. Binding of iRBC to EPRC through the CIDR domain of PfEMP1 blocks protein C activation and leads to highly localised coagulopathy (Turner et al., 2013). It is hypothesised that whilst EPRC mediated cytoadherecne will take place in many vascular beds, the localised coagulopathy is particularly likely to occur in the brain because of low constitutive expression of ERCP and TM (Moxon et al., 2013). The fact that the PfEMP1 variants able to bind to EPRC are those that have been associated with severe malaria (Turner et al., 2013) provides a potentially important unifying link between parasite and host factors mediating severity.

Interaction with other infections

Malaria is particularly a problem of vulnerable communities exposed to multiple and concurrent health risks which often interact. HIV infection is a major risk factor for severe

disease and death due to malaria in both children and adults and malaria in pregnant women is associated with an increased risk of HIV transmission to the foetus (van Eijk et al., 2007). The most important interactions from a public health point of view are with invasive bacterial disease. In most series of African children with severe malaria the prevalence of concurrent invasive bacterial disease is far in excess of that which could occur by chance (Church and Maitland, 2014). Importantly, case fatality is much higher in those with dual infections. A number of mechanisms for the association have been proposed including translocation of gram negative organisms across leaky bowel, specific macrophage dysfunction and functional hyposplenism (Gomez-Perez et al., 2014). It is estimated that in some areas malaria may be responsible for 50% of all episodes of IBD (Scott et al., 2011) leading to the counterintuitive idea that malaria may cause more deaths indirectly than it does directly. This idea is strongly supported by massive reductions in all cause childhood mortality over a very short time period concurrent with aggressive malaria control (Kleinschmidt et al., 2009).

Malaria in pregnancy

In non-immune populations, malaria in pregnancy may be associated with still birth and severe disease in the mother with especially high risk of hypoglycaemia . In endemic areas, where the populations develops a degree of immunity to malaria, infection leads to maternal anaemia and low birth weight of the child (McLean et al., 2015). Whilst this is sometimes characterised as loss of immunity, this is not strictly the case; rather the presence of the placenta offers a new site for parasite sequestration through the selection of parasites able to bind to CSA on the syncitiotrophoblast (Khunrae et al., 2010). The increased incidence and density of peripheral parasites observed in pregnancy represents spill over from a new 'privileged' site in a subject who retains previously effective immune responses. Over successive pregnancies women develop antibodies directed at the specific PfEMP1 variants involved in CSA mediated cytoadherence (Ataide et al., 2014) and women in later pregnancies are significantly protected against the ill effects of infection.

Severe malaria due to P. vivax

Historically *P. vivax* has been considered a relatively benign parasite. This idea was supported by the very low incidence of deaths in P vivax in international travellers and minimal or no deaths reported from some geographical areas (e.g. (Luxemburger et al., 1997)). However, this view is increasingly challenged by a large number of studies reporting significant rates of severe disease and death. In children in high transmission areas the commonest reported

syndrome of severity is severe anaemia, whereas in adults under lower transmission it is multi organ failure (Rahimi et al., 2014). Two recent comprehensive analyses of published literature (Baird, 2013; Naing et al., 2014) came to the conclusion that rates of severe disease and death are broadly comparable in the two parasites. However, there are substantial difficulties in interpreting many studies: differential diagnoses is often challenging and hospital based studies may lead to over estimates of rates (Rahimi et al., 2014). None the less although more high quality epidemiological and clinical studies are required it seems clear that the idea of *P. vivax* being in any sense a benign parasite cannot be sustained.

The fact that *P. vivax* causes significant amounts of severe disease and death (whatever the exact comparative figures) also raises critical questions about the pathogenesis of both *P. vivax* and *P. falciparum*. As *P. vivax* only achieves low parasitaemias and does not undergo extensive intravascular sequestration what does this mean for the idea that biomass and microvascular obstruction are key in *P. falciparum*? There is a dearth of modern autopsy studies in *P. vivax* but in a recent exception (Lacerda et al., 2012) in 17 deaths attributed to *P. vivax* in Brazil, 13 were considered at autopsy to be due to the parasite. The most common finding was of respiratory pathology with pulmonary oedema and multi organ failure, though there was no evidence of intravascular sequestration. Recently however Baird has hypothesised that a large part of the biomass may in fact be sequestered in extravascular spaces in haemopoeitic tissue (Baird, 2013). There is an urgent need for high quality studies of the similarities and differences in both the clinical spectrum and associated pathogenic factors in severe malaria due to *P. vivax* and *P. falciparum*.

Antimalarials, resistance and new drug targets

The use of antimalarial drugs has been the mainstay of control and prophylaxis against malaria since the first use of quinine from the cinchona tree. Many drugs were developed against malaria in the 20th century with the most important being chloroquine and artemisinin derivatives However, the ability of, in particular *P. falciparum*, to develop resistance to these treatments has threatened their continuing efficacy and raised the importance of the use of combinations as well as developing new drugs and novel targets. In response to the spread of parasite resistance to chloroquine, and other antimalarials such as pyrimethamine and sulfadoxine, artemisinin-based combination therapies (ACTs) are now recommended for treatment of uncomplicated malaria and their use has been an important part of the remarkable decrease in malaria globally. However, there were reports from Cambodia of decreasing sensitivity of *P. falciparum* to artemisinin derivatives where patients had significantly longer

clearance times after monotherapy (Dondorp et al., 2009; Noedl et al., 2008) and this has developed further into decreasing clinical efficacy (Ashley et al., 2014a; Guerin et al., 2009; Slater et al., 2016) and potential partner drug resistance (Menard et al., 2015). This has become a potential threat to the continued efficacy of ACTs and programs have been developed in an attempt to contain and eliminate ART resistance.

Mechanism of artemisinin action and resistance

Whilst it is not understood how artemisinin kills the malaria parasite it appears that opening of the endoperoxide ring is essential and that this requires heme (arising from the degradation of hemoglobin) resulting in an active species that reacts with parasite cellular components resulting in parasite death. Recent work has supported this hypothesis as artemisinin antiparasite activity through increased oxidative stress in the parasite cytoplasm requires hemoglogin digestion (Haynes et al., 2012; Klonis et al., 2011).

There is now some insight developing with respect to the mechanism of resistance for artemisinin by *P. falciparum* through genome wide association studies and gene candidate based approaches (Ariey et al., 2014; Dogovski et al., 2015; Mbengue et al., 2015; Mok et al., 2015; Straimer et al., 2015). Genome sequencing of parasites from patients treated with ACT that demonstrated reduced parasite clearance rates identified mutations in the gene encoding a K-13 propeller protein suggesting that this protein was an important determinant of artemisinin resistance (Ariey et al., 2014; Ashley et al., 2014a; Popovici et al., 2015; Takala-Harrison et al., 2015). Insertion of specific mutations identified into the K13 locus conferred an increased resistance on specific parasite lines not only showing that this gene was involved in the slow clearance phenotype for artemisinin but also suggesting that it was a complex phenotype for which other genes made a contribution to the overall phenotype (Straimer et al., 2015). Whilst it is clear that Pfkelch13 is an excellent marker for artemisinin resistance in *P. falciparum* many polymorphisms have been identified in the corresponding gene and it is not clear what role all of these play in directly conferring artemisinin resistance (Miotto et al., 2015).

Transcriptional analysis of parasites from patients with acute malaria showed that artemisinin resistance was associated with an increase in the unfolded protein response and that parasites exhibited a slow development phase in the early stages of the blood stage life cycle and this helps mitigate the damage caused by artemisinin (Mok et al., 2015). This is consistent with other work that showed artemisinin induced a cell stress response resulting in accumulation of ubiquitinated proteins and increased activity of the proteasome (Dogovski et al., 2015). Resistant parasites have an enhanced cell stress response resulting in lower levels of

ubiquitinated proteins and a delayed onset of cell death. It has also been suggested that phosphatidylinositol-3-kinase (PfPI3K) is a molecular target of artemisinin and is involved in mediating the resistance mechanism (Mbengue et al., 2015). They showed that artemisinin and its derivatives can inhibit PfPI3K and that this would result in decreased phosphatidylinositol-3-phosphate (PI3P) and as a result decreased export of virulence proteins to the infected erythrocyte. They hypothesize that mutations in PfKelch13 reduce both its binding to and polyubiquitination of PfPI3K thus increasing levels of the protein due to decreased degradation. This occurs by recruitment of a ubiquitin ligase to PfPI3K and as a result decreased PI3P and decreased protein export. However, they have suggested that the role of PfPI3K in artemisinin resistance may not be linked to alterations in protein export but to reduced activity of the PfPI3K-AKT signal transduction pathway. Whilst there is some tantalizing new information on the mechanism of resistance of *P. falciparum* to artemisinin there is some inconsistency in current models and it will be important to show that PfPI3K–AKT mediate the phenotype encoded by the PfKelch13 mutations in the *P. falciparum*.

Development of novel antimalarials

Currently, there are effective drugs to treat and control both vivax and falciparum malaria, however, development of resistance to these including artemisinin and its derivatives for *P. falciparum* has raised serious concerns about their long term utility. Additionally, as *P. vivax* and *P. ovale* form dormant liver stages (hypnozoites) they are responsible for a significant number of relapse infections and currently there is only one drug, primaquine, that can provide a radical cure. This is particularly concerning as elimination of malaria is a major global health priority and the current suite of antimalarial drugs are unlikely to be sufficient for this task. Therefore, in order to progress the malaria elimination agenda it has become important to develop new therapeutics that act broadly to cure the asexual blood stage to alleviate symptoms, clear the liver stage that can result in relapses for *P. vivax* and *P. ovale* malaria and block transmission. This has resulted in the development of public-private partnerships that have fostered the use of medicinal chemistry-based approaches as well as novel high throughput drug screens and platforms to identify new chemical entities and targets that are showing promise in preclinical and clinical testing. This has been increased by the availability of the Tres Cantos antimalarial set (TCAMS) of 13,000 compounds that have antimalarial activity (Gamo et al., 2010). From this Medicines for Malaria Venture (MMV) assembled 400 unique compounds with blood stage antimalarial activity that have been called the malaria box (Spangenberg et al., 2013).

Using whole cell screens of the asexual blood stage of *P. falciparum* the spiroindolone class of compounds was identified leading to a drug candidate (KAE609, cipargamin) that has been progressed to clinical trials with positive results (Leong et al., 2014; Rottmann et al., 2010; Stein et al., 2015; White et al., 2014b). Cipargamin and related compounds target the P-type Na^+-ATPase (PfATP4) expressed on the plasma membrane of the parasite by disruption of sodium homeostasis resulting in the blocking of asexual blood stage development and transmission to the mosquito (Spillman et al., 2013). PfATP4 has proven to be a target for a number of chemical classes including the pyrazoleamides (Vaidya et al., 2014). Additionally, a high throughput screen identified the orally available dihydroisoquinolones resulting in the clinical candidate (+)-SJ733 which is a potent inhibitor of blood stage and transmission stages of malaria parasites (Jimenez-Diaz et al., 2014). This class of compounds also targets PfATP4 and indicates that disruption of sodium homeostasis is an excellent target for novel antimalarial drugs.

Whole cell screening approaches have also identified a 2,6-disubstituted quinoline-4-carboxamide scaffold that lead to development of DDD107498 that targets translation elongation factor 2 (eEF2) of *P. falciparum* and is active against multiple life-cycle stages (Baragana et al., 2015). Additionally, a cell based screen has identified the imidazopyrazine class of compounds that target the lipid kinase, phosphatidylinositol 4-kinase (PI4K) (McNamara et al., 2013). The imidazopyrazines interfere with the ATP-binding pocket of PI4K and as a consequence alter the intracellular distribution of phosphatidylinositol 4-phosphate. These compounds inhibit multiple stages of *P. vivax* and *P. falciparum* and have also been shown to inhibit liver hypnozoites in *P. cynomolgi* (McNamara et al., 2013; Zeeman et al., 2014). They also block mosquito transmission in mouse models of malaria. Screening for compounds that block development of the liver stage of *P. falciparum* has identified the imidazolopiperazine class of compounds which are orally available with potent blood and transmission stage activity and this has led to development of the clinical candidate KAF156 (Kuhen et al., 2014). The exact mechanism of action of this class of compounds is unknown but selection of parasite resistance has identified the cyclic amine resistance gene (Pf3D7_0321900), however, it is not known if this is the actual target or a resistance mechanism.

Whilst screening of whole cells has been successful in identifying new classes of compounds as potential antimalarials synthetic approaches have also been important and this has produced the ozonides which retain the endoperoxide bridge that is present in artemisinin and the mechanism of action is similar to that of artemisinin where the peroxide bond is broken

by heme and ferrous iron thus producing free radicals that cross-link proteins and other cellular components (Dong et al., 2010). Whilst the mechanism of activation for ozonides appears to be the same as artemisinin the specific targets for parasitic activity appear to be different. There are a number of ozonides that have been developed including the recently marketed Synriam and OZ439 which is currently in clinical trials (Vennerstrom et al., 2004).

Additionally, there has been considerable efforts in developing lead candidates against known drug targets. This has included the mitochondrial enzyme dihydroorotate dehydrogenase (DHOD) for which the triazolopyrimidines, a new chemical class of *P. falciparum* inhibitors, were identified (Kokkonda et al., 2016). From this DSM265 has been identified that shows potent levels of activity in humanized mouse malaria models.

The development of new treatments for the radical cure of *P. vivax* and *P. ovale* is important as the current treatment is primaquine, which has potential toxic properties in patients with glucose-6-phophate deficiency to produce hemolytic anemia (reviewed in (Ashley et al., 2014b)). Screening for hypnozoite active compounds has been difficult, but as discussed above, some screens have been performed using the *P. cynomolgi* model in rhesus monkeys to show that the imidazopyrazine KAI407 is active against this dormant form raising the prospect of new improved treatments (Zeeman et al., 2014). However, it has recently been shown that liver chimeric humanized mice can support the growth of *P. vivax* infection along with the persistence of hypnozoites and this has been an important technical step forward (Mikolajczak et al., 2015). Added to this is the development of human liver microscale platforms that support the growth and development of hepatic stages for both *P. falciparum* and *P. vivax* (March et al., 2013). These platforms have added considerably to the possibility of screening for new inhibitors of *P. vivax* and the hypnozoite form.

Whilst it is important that new alternatives to primaquine be identified the potential to use low dose regimens of this drug (Bancone et al., 2016) as well as the development of the 8-aminoquinoline tafenoquine in the early 1990's has provided potential alternatives. Tafenoquine is significantly more potent than primaquine and is currently in phase III clinical trials and shows promise as an alternative (Llanos-Cuentas et al., 2014).

Current status for Vaccine Development

It is widely accepted that new tools are required if we are to maintain recently achieved levels of disease control and move towards elimination and eventual global eradication of malaria. Efforts to develop vaccines over more than thirty years have targeted pre erythrocytic (sporozoite and liver stages), blood stages and sexual stages. An effective pre erythroctytic

vaccine would prevent disease by preventing the establishment of blood stage infection but because immunity is stage specific, any breakthrough would leave the subject at risk of disease. Vaccines that target the replicating asexual blood stages have been considered important to control morbidity and mortality. Vaccines against the sexual stages would interrupt the transmission cycle but not have a direct effect on an established infection in the vaccinee.

As the idea of elimination has gained ground, emphasis in vaccine development has move towards the pre erythrocytic and sexual stages, predicated on the bottleneck hypothesis: this supposes that attacking the numerical bottlenecks in parasite development; the injected sporozoites prior to liver invasion and mosquito midgut transmission stages (Figure 5) - will lead to increased vaccine efficacy compared to one targeting the replicating asexual blood stages that are present in much higher numbers and have evolved a variety of immune evasion mechanisms for long term survival in the circulation. The problem with this strategy is that if a single sporozoite or ookinete breaks through then the pathogenic replicative cycle continues since the immunity generated against these targets appears to be strictly stage-specific (Felgner et al., 2013). This will become even more important as we move towards elimination. A combination of population loss of naturally acquired blood stage immunity with waning vaccine efficacy in the context of drug resistance would be a very dangerous situation for rebound of malaria transmission.

With this in mind, what would the ideal malaria vaccine look like? Ideal vaccines would be based on conserved targets that induce lifelong sterile protection early in life with as few doses as possible. So far, this has not been achievable for a malaria vaccine. The most advanced vaccine to date, the RTS,S/AS01 anti-sporozoite vaccine, having progressed through Phase III clinical trials involving over 15,000 African children, is the gold standard against which all future vaccines will be assessed. After a year of follow-up, vaccination with a three-dose series reduced clinical malaria cases by 28% in young children and 18% in infants (Greenwood and Doumbo, 2016). A key finding of these trials is the short duration of protection. Efforts to understand why this is the case are urgently required. A comprehensive study of interaction between malaria parasites and the human immune system and how they co-evolve is another area that will help in designing better vaccine strategies.

Whilst there has been much focus on sub unit vaccines, there is a renewal of interest in whole cell vaccines .Attenuated live cell sporozoite vaccine, (PfSPZ) have delivered sterile protection (no blood stage development) against homologous challenge (injection with sporozoites of the same parasite strain) in early Phase I/IIa clinical trials (Epstein et al., 2011; Seder et al., 2013). However, this vaccine has major drawbacks that will need to be overcome,

including the lack of demonstrated heterologous (cross strain) protection, the high numbers of parasites and intravenous route of delivery required to induce anti-sporozoite immunity in volunteers, and the logistical requirement for a liquid nitrogen cold chain to maintain viability of the vaccine. Other live cell sporozoite vaccine strategies, such as genetic attenuation of parasites (Mikolajczak et al., 2014; Spring et al., 2013; van Schaijk et al., 2014) which may be beneficial in terms of quality control, and CPS (chemoprophylaxis with sporozoites), where much lower numbers of parasites are needed to induce immunity (Roestenberg et al., 2009), but requires chloroquine treatment following each vaccination dose, may have incremental improvements over PfSPZ but all share the fundamental challenge of inducing strain-transcendent protection, a top priority for vaccine development.

A promising strategy may be targeting combinations of antigens expressed on multiple life cycle stages (Hill, 2011; Theisen et al., 2014). Assuming we can overcome the issue of longevity of immune responses, the pros of this approach are that addition of a blood stage component to an anti-sporozoite target would reduce the risk of morbidity and mortality and provide immune protection while transmission declines, providing insurance against epidemics of severe disease should transmission rebound due to other intervention failures.

Leading multi-stage vaccine candidate antigens are CSP from sporozoites (Cohen et al., 2010), PfRh5 on the merozoite (Douglas et al., 2015), Pfs25, 48/45 & 230 on midgut stages (reviewed in (Nikolaeva et al., 2015)) and AMA-1, expressed on both sporozoite (Silvie et al., 2004) and merozoites (Peterson et al., 1989). However, the failure of AMA-1 based vaccines (Payne et al., 2016; Spring et al., 2009) to provide homologous protection against either sporozoite or blood stage challenge is a concern.

Vaccine platforms

Subunit, live cell, DNA and viral-vectored approaches each have their pros and cons. Most commercial vaccines are based on attenuated live cells, which have the benefit of displaying a range of antigens in their native conformation to the immune system. Research and development of attenuated sporozoite (Mikolajczak et al., 2014; Spring et al., 2013; van Schaijk et al., 2014) and blood stage (Good et al., 2013) malaria vaccines continues. Viral platforms (Reyes-Sandoval et al., 2012) are superior inducers of cell-mediated immunity and have the additional benefit of being able to express several proteins from the same vector, making them attractive platforms for multi-target vaccines (Bauza et al., 2015). Safety concerns around the generation of or pre-existing immunity to the virus have been addressed by using non-human tropic viruses as vaccine vectors. Viral-vectored products comprise the

majority of the current list of malaria vaccines in clinical development (http://www.who.int/immunization/research/development/Rainbow_tables/en/). Subunit vaccines are preferred for their good safety and tolerability profiles but require powerful adjuvants for optimal responses.

The complexity of *Plasmodium* is the biggest challenge to effective vaccine design. As discussed above, many antigens are highly polymorphic, and those that are conserved may be poorly immunogenic. Interrogation of the genome in combination with high throughput -omics technologies (Doolan et al., 2008) and immune profiling with sera from patients with clearly defined disease outcomes (Osier et al., 2014; Richards et al., 2013) are being used to compile lists of potential new targets. This output combined with functional genomics to identify essential parasite proteins will be a helpful step in ranking the thousands of potential targets for vaccine suitability.

Control and elimination of malaria historical perspective and prospects for the future

The first half of the 20th century saw remarkable progress in understanding the biology and epidemiology of malaria and applying this to efforts to control the disease, particularly at the geographical boundaries of transmission. The discovery of DDT's insecticidal properties in 1939 provided a powerful new tool for attacking the vectors and interrupting transmission. In 1955 WHO called for a global campaign of eradication. Much has been written about its failures, most notably in Africa. However, there were also extraordinary successes and malaria was eliminated from large parts of the world. Perhaps the most important lesson came from the near successes: in some areas enormous reductions were achieved but the last stages of elimination are by far the hardest and when progress stalled there was massive rebound. This provides a frightening warning of what we may face if we are successful but not totally successful in current efforts, especially in areas with inherently high transmission potential.

Few systematic attempts at control were made in Africa and throughout 1980' and 90's the situation grew progressively worse, largely attributed to the widespread development of resistance to chloroquine, then the mainstay across Africa for the treatment of malaria. During this period malaria attributable mortality probably doubled and by the end of the twentieth century, malaria in Africa was appropriately described as a disaster, with apparent paralysis of national and international will to tackle the situation. Eventually however there was action and remarkably between 2000 and 2008 total global spending on malaria control rose tenfold. Much of this was spent on two key interventions, insecticide treated bed nets and effective new

approaches to antimalarial therapy based on the combination of artemisinins with partner drugs . Since 2000 global malaria death rates due to malaria have fallen by 60% and in Africa by 66% (WHO, 2015). However, as is often the case, things are not as simple as they seem. In several well documented cases it is clear that malaria transmission has been falling steadily over a long period of time and many of the reductions preceded by a considerable time the introduction of new interventions (O'Meara et al., 2008). None of this argues against the critical importance of increasing investment in control but it does mean that there is much about the epidemiology on a continental scale what we still do not understand.

Over the last few years there has been a renewed interest in the idea of malaria eradication as the necessary goal of global efforts. In 2015 a new Global Technical Strategy for Malaria developed by WHO and partners was adopted by the World Health Assembly (WHO, 2015) which is based on the aim of accelerating efforts with eradication as the long term goal but with a realistic appreciation of the enormous barriers which must be overcome and the threats to current gains, especially the development of artemisinin resistance and widespread insecticide resistance. It remains the case that for the vast majority of endemic areas control of morbidity and mortality is the first priority and an absolute requisite before elimination can be considered. Few would consider the eradication of malaria possible in any reasonable time scale using current tools. The hope of elimination and eventual eradication must rest on the development of radical new tools, whether it be drugs, vaccines or genetically modified vectors, and this will require major investment in research to exploit our knowledge of the parasites basic biology and epidemiology.

Figure Legends

Figure 1. Life Cycle of *P. falciparum*

Malaria infection is initiated with the injection of sporozoites (spz) into the dermis by a feeding female anopheline mosquito (1). Within minutes of deposition, spz enter the vasculature and are transported to the liver, where they exit the sinusoids through Kupffer or endothelial cells and pass into a hepatocyte (2). Active invasion is preceded by a period of cellular traversal until a suitable hepatocyte is found. There, they form a PVM and undergo a period of schizogony until 10s of 1000s of daughter merozoites are released in packets of merosomes into the liver vasculature. There they encounter erythrocytes and begin a chronic cycle of

asexual schizogony in the bloodstream (3). A proportion of asexually reproducing merozoites are reprogrammed to undergo gametocytogenesis (4). Developing gametocytes sequester in the bone marrow for 15 days and are present in the peripheral circulation until taken up by a feeding mosquito where they emerge as extracellular male and female gametes in the midgut (5). Mating occurs by fusion of micro and macrogamete to form a zygote that transforms over the next 24 hours into a motile ookinete that migrates through the mosquito midgut epithelium and encysts to become an oocyst where asexual sporogenic phase of replication occurs (6). Motile sporozoites are released into the haemocoel by oocyst rupture and pass into the salivary glands where they can be injected into the next human host.

Figure 2. Merozoite Invasion of Erythrocytes

Initial contact between merozoite and erythrocyte is mediated by low-affinity interactions between red cell and merozoite surface coat proteins (1) and can involve wrapping of the erythrocyte membrane around the body of the merozoite such that reorientation occurs, bringing the apical end of the merozoite into direct contact with the erythrocyte membrane. A specific ligand-receptor interaction follows (2) mediated by proteins of the EBA and PfRh family members. This initiates downstream invasion events involving the Rh5 protein complex binding to the host cell receptor basigin (3) which initiates a calcium flux into the erythrocyte and signalling microneme secretion allowing deposition of the RON complex into the erythrocyte membrane allowing AMA-1 to bind directly forming a moving junction (4). By the reverse mobilization of a merozoite-internal acto-myosin complex, the merozoite pushes into the erythrocyte membrane. This is concurrent with discharge of rhoptry contents that contribute to formation of a parasitophorous vacuole membrane (PVM) that will enclose the merozoite (5,6,7). Upon sealing of the PVM and the erythrocyte plasma membrane a period of echinocytosis occurs due to loss of water from the erythrocyte cytosol (8). Recovery of erythrocyte homeostasis follows (9).

Figure 3. Protein export induced changes in the host erythrocyte

Following invasion, the parasite begins to export hundreds of proteins into the host cell. These proteins must travel via the vesicle mediated classical secretory pathway arising at the endoplasmic reticulum (ER), across the PM and into the PV. At the PV membrane (PVM) a protein translocon termed the PTEX mediates the movement of proteins destined for export into the host cell. Trafficking within the host cell is mediated by parasite derived vesicular structures such as EDVs, J-dots or membranous organelles known as Maurer's clefts. The

ultimate destination of these proteins can be the erythrocyte cytosol, cytoskeleton or membrane. The exported protein KAHRP, among others, induces elevation of points at the erythrocyte membrane known as knobs from which the major virulence factor PfEMP1 is displayed to the extracellular environment. From this point, PfEMP1 mediates cytoadhesion to endothelial cell receptors and is responsible for the major symptoms of malaria. Exported parasite proteins such as RESA also induce the dramatic restructuring of the erythrocyte cytoskeleton in order to withstand higher temperatures and shear stress. New permeability pathways (NPP/PSAC) are also established at the erythrocyte membrane allowing the parasite to scavenge nutrients and expel waste products. Abbreviations: ER, endoplasmic reticulum; PM, parasite membrane; PV, parasitophorous vacuole; PVM, parasitophorous vacuole membrane ; PTEX, Plasmodium Translocon of Exported proteins; EDV, electron dense vesicles; KAHRP, knob-associated histidine-rich protein; RESA, ring exported surface antigen; PfEMP1, *Plasmodium falciparum* erythrocyte membrane protein 1.

Figure 4. Schematic illustrating the development of immunity to different manifestations of malaria infection in an endemic population.

The age distribution of severe, mild and asymptomatic infections is shown as a percentage of the maximum period prevalence achieved.

Figure 5. Vaccine-targetable processes within the malaria lifecycle.

A malaria vaccine could potentially intervene at many different points in the life cycle (right panel). The panel on the left corresponds to parasite population dynamics in individual hosts. Numerical bottlenecks occurring at the points of sporozoite migration through the skin and ookinete penetration of the midgut are considered priority areas for vaccine development.

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References

Adams, J.H., Sim, B.K.L., Dolan, S.A., Fang, X., Kaslow, D.C., and Miller, L.H. (1992). A family of erythrocyte binding proteins of malaria parasites. Proc Natl Acad Sci USA *89*, 7085-7089.

Ahmed, M.A., and Cox-Singh, J. (2015). - an emerging pathogen. ISBT Sci Ser *10*, 134-140.

Alexander, D.L., Mital, J., Ward, G.E., Bradley, P., and Boothroyd, J.C. (2005). Identification of the moving junction complex of *Toxoplasma gondii:* a collaboration between distinct secretory organelles. PLoS Pathog *1*, e17.

Amino, R., Giovannini, D., Thiberge, S., Gueirard, P., Boisson, B., Dubremetz, J.F., Prevost, M.C., Ishino, T., Yuda, M., and Menard, R. (2008). Host cell traversal is important for progression of the malaria parasite through the dermis to the liver. Cell host & microbe *3*, 88-96.

Amino, R., Thiberge, S., Martin, B., Celli, S., Shorte, S., Frischknecht, F., and Menard, R. (2006). Quantitative imaging of *Plasmodium* transmission from mosquito to mammal. Nat Med *12*, 220-224.

Ariey, F., Witkowski, B., Amaratunga, C., Beghain, J., Langlois, A.C., Khim, N., Kim, S., Duru, V., Bouchier, C., Ma, L., *et al.* (2014). A molecular marker of artemisinin-resistant *Plasmodium falciparum* malaria. Nature *505*, 50-55.

Ashley, E.A., Dhorda, M., Fairhurst, R.M., Amaratunga, C., Lim, P., Suon, S., Sreng, S., Anderson, J.M., Mao, S., Sam, B., *et al.* (2014a). Spread of artemisinin resistance in *Plasmodium falciparum* malaria. N Engl J Med *371*, 411-423.

Ashley, E.A., Recht, J., and White, N.J. (2014b). Primaquine: the risks and the benefits. Malaria journal *13*, 418.

Ataide, R., Mayor, A., and Rogerson, S.J. (2014). Malaria, primigravidae, and antibodies: knowledge gained and future perspectives. Trends Parasitol *30*, 85-94.

Baer, K., Roosevelt, M., Clarkson, A.B., Jr., van Rooijen, N., Schnieder, T., and Frevert, U. (2007). Kupffer cells are obligatory for *Plasmodium yoelii* sporozoite infection of the liver. Cell Microbiol *9*, 397-412.

Baird, J.K. (2013). Evidence and implications of mortality associated with acute *Plasmodium vivax* malaria. Clin Microbiol Rev *26*, 36-57.

Baldwin, M.R., Li, X., Hanada, T., Liu, S.C., and Chishti, A.H. (2015). Merozoite surface protein 1 recognition of host glycophorin A mediates malaria parasite invasion of red blood cells. Blood *125*, 2704-2711.

Bancone, G., Chowwiwat, N., Somsakchaicharoen, R., Poodpanya, L., Moo, P.K., Gornsawun, G., Kajeechiwa, L., Thwin, M.M., Rakthinthong, S., Nosten, S., *et al.* (2016). Single Low Dose Primaquine (0.25mg/kg) Does Not Cause Clinically Significant Haemolysis in G6PD Deficient Subjects. PloS one *11*, e0151898.

Baragana, B., Hallyburton, I., Lee, M.C., Norcross, N.R., Grimaldi, R., Otto, T.D., Proto, W.R., Blagborough, A.M., Meister, S., Wirjanata, G., *et al.* (2015). A novel multiple-stage antimalarial agent that inhibits protein synthesis. Nature *522*, 315-320.

Bargieri, D.Y., Andenmatten, N., Lagal, V., Thiberge, S., Whitelaw, J.A., Tardieux, I., Meissner, M., and Menard, R. (2013). Apical membrane antigen 1 mediates apicomplexan parasite attachment but is dispensable for host cell invasion. Nat Commun *4*, 2552.

Baruch, D.I., Pasloske, B.L., Singh, H.B., Bi, X., Ma, X.C., Feldman, M., Taraschi, T.F., and Howard, R.J. (1995). Cloning the *P. falciparum* gene encoding PfEMP1, a malarial variant antigen and adherence receptor on the surface of parasitized human erythrocytes. Cell *82*, 77-87.

Baum, J., Chen, L., Healer, J., Lopaticki, S., Boyle, M., Triglia, T., Ehlgen, F., Ralph, S.A., Beeson, J.G., and Cowman, A.F. (2009). Reticulocyte-binding protein homologue 5 - an

essential adhesin involved in invasion of human erythrocytes by *Plasmodium falciparum*. Int J Parasitol *39*, 371-380.

Bauza, K., Atcheson, E., Malinauskas, T., Blagborough, A.M., and Reyes-Sandoval, A. (2015). Tailoring a Combination Preerythrocytic Malaria Vaccine. Infect Immun *84*, 622-634.

Besteiro, S., Dubremetz, J.F., and Lebrun, M. (2011). The moving junction of apicomplexan parasites: a key structure for invasion. Cell Microbiol *13*, 797-805.

Besteiro, S., Michelin, A., Poncet, J., Dubremetz, J.F., and Lebrun, M. (2009). Export of a *Toxoplasma gondii* rhoptry neck protein complex at the host cell membrane to form the moving junction during invasion. PLoS Pathog *5*, e1000309.

Bhanot, P., Schauer, K., Coppens, I., and Nussenzweig, V. (2005). A surface phospholipase is involved in the migration of plasmodium sporozoites through cells. J Biol Chem *280*, 6752-6760.

Boddey, J.A., and Cowman, A.F. (2013). *Plasmodium* nesting: remaking the erythrocyte from the inside out. Annu Rev Microbiol *67*, 243-269.

Bosch, J., Buscaglia, C.A., Krumm, B., Ingason, B.P., Lucas, R., Roach, C., Cardozo, T., Nussenzweig, V., and Hol, W.G. (2007). Aldolase provides an unusual binding site for thrombospondin-related anonymous protein in the invasion machinery of the malaria parasite. Proceedings of the National Academy of Sciences of the United States of America *104*, 7015-7020.

Bull, P.C., Lowe, B.S., Kortok, M., Molyneux, C.S., Newbold, C.I., and Marsh, K. (1998). Parasite antigens on the infected red cell surface are targets for naturally acquired immunity to malaria. Nature Med *4*, 358-360.

Chan, J.A., Fowkes, F.J., and Beeson, J.G. (2014). Surface antigens of *Plasmodium falciparum*-infected erythrocytes as immune targets and malaria vaccine candidates. Cell Mol Life Sci *71*, 3633-3657.

Chan, J.A., Howell, K.B., Reiling, L., Ataide, R., Mackintosh, C.L., Fowkes, F.J., Petter, M., Chesson, J.M., Langer, C., Warimwe, G.M., *et al.* (2012). Targets of antibodies against *Plasmodium falciparum*-infected erythrocytes in malaria immunity. J Clin Invest *122*, 3227-3238.

Chen, L., Lopaticki, S., Riglar, D.T., Dekiwadia, C., Uboldi, A.D., Tham, W.H., O'Neill, M.T., Richard, D., Baum, J., Ralph, S.A., *et al.* (2011). An EGF-like protein forms a complex with PfRh5 and is required for invasion of human erythrocytes by *Plasmodium falciparum*. PLoS Pathog *7*, e1002199.

Church, J., and Maitland, K. (2014). Invasive bacterial co-infection in African children with *Plasmodium falciparum* malaria: a systematic review. BMC Med *12*, 31.

Cohen, J., Nussenzweig, V., Nussenzweig, R., Vekemans, J., and Leach, A. (2010). From the circumsporozoite protein to the RTS, S/AS candidate vaccine. Hum Vaccin *6*, 90-96.

Cohen, S., McGregor, I.A., and Carrington, S.C. (1961). Gamma-globulin and acquired immunity to human malaria. Nature *192*, 733-737.

Collins, C.R., Hackett, F., Strath, M., Penzo, M., Withers-Martinez, C., Baker, D.A., and Blackman, M.J. (2013). Malaria parasite cGMP-dependent protein kinase regulates blood stage merozoite secretory organelle discharge and egress. PLoS Pathog *9*, e1003344.

Collins, W.E., and Jeffery, G.M. (1999). A retrospective examination of sporozoite- and trophozoite-induced infections with *Plasmodium falciparum*: development of parasitologic and clinical immunity during primary infection. Am J Trop Med Hyg *61*, 4-19.

Coppi, A., Natarajan, R., Pradel, G., Bennett, B.L., James, E.R., Roggero, M.A., Corradin, G., Persson, C., Tewari, R., and Sinnis, P. (2011). The malaria circumsporozoite protein has two functional domains, each with distinct roles as sporozoites journey from mosquito to mammalian host. J Exp Med *208*, 341-356.

Coppi, A., Tewari, R., Bishop, J.R., Bennett, B.L., Lawrence, R., Esko, J.D., Billker, O., and Sinnis, P. (2007). Heparan sulfate proteoglycans provide a signal to Plasmodium sporozoites to stop migrating and productively invade host cells. Cell host & microbe *2*, 316-327.

Crompton, P.D., Kayala, M.A., Traore, B., Kayentao, K., Ongoiba, A., Weiss, G.E., Molina, D.M., Burk, C.R., Waisberg, M., Jasinskas, A., *et al.* (2010). A prospective analysis of the Ab response to *Plasmodium falciparum* before and after a malaria season by protein microarray. Proceedings of the National Academy of Sciences of the United States of America *107*, 6958-6963.

Crosnier, C., Bustamante, L.Y., Bartholdson, S.J., Bei, A.K., Theron, M., Uchikawa, M., Mboup, S., Ndir, O., Kwiatkowski, D.P., Duraisingh, M.T., *et al.* (2011). Basigin is a receptor essential for erythrocyte invasion by *Plasmodium falciparum*. Nature *480*, 534-537.

Cunnington, A.J., Walther, M., and Riley, E.M. (2013). Piecing together the puzzle of severe malaria. Sci Transl Med *5*, 211ps218.

Das, S., Hertrich, N., Perrin, A.J., Withers-Martinez, C., Collins, C.R., Jones, M.L., Watermeyer, J.M., Fobes, E.T., Martin, S.R., Saibil, H.R., *et al.* (2015). Processing of *Plasmodium falciparum* Merozoite Surface Protein MSP1 Activates a Spectrin-Binding Function Enabling Parasite Egress from RBCs. Cell host & microbe *18*, 433-444.

Dogovski, C., Xie, S.C., Burgio, G., Bridgford, J., Mok, S., McCaw, J.M., Chotivanich, K., Kenny, S., Gnadig, N., Straimer, J., *et al.* (2015). Targeting the cell stress response of *Plasmodium falciparum* to overcome artemisinin resistance. PLoS Biol *13*, e1002132.

Dondorp, A.M., Ince, C., Charunwatthana, P., Hanson, J., van Kuijen, A., Faiz, M.A., Rahman, M.R., Hasan, M., Bin Yunus, E., Ghose, A., *et al.* (2008). Direct *in vivo* assessment of microcirculatory dysfunction in severe falciparum malaria. J Infect Dis *197*, 79-84.

Dondorp, A.M., Nosten, F., Yi, P., Das, D., Phyo, A.P., Tarning, J., Lwin, K.M., Ariey, F., Hanpithakpong, W., Lee, S.J., *et al.* (2009). Artemisinin resistance in *Plasmodium falciparum* malaria. N Engl J Med *361*, 455-467.

Dong, Y., Wittlin, S., Sriraghavan, K., Chollet, J., Charman, S.A., Charman, W.N., Scheurer, C., Urwyler, H., Santo Tomas, J., Snyder, C., *et al.* (2010). The structure-activity relationship of the antimalarial ozonide arterolane (OZ277). J Med Chem *53*, 481-491.

Doolan, D.L., Mu, Y., Unal, B., Sundaresh, S., Hirst, S., Valdez, C., Randall, A., Molina, D., Liang, X., Freilich, D.A., *et al.* (2008). Profiling humoral immune responses to *P. falciparum* infection with protein microarrays. Proteomics *8*, 4680-4694.

Douglas, A.D., Baldeviano, G.C., Lucas, C.M., Lugo-Roman, L.A., Crosnier, C., Bartholdson, S.J., Diouf, A., Miura, K., Lambert, L.E., Ventocilla, J.A., *et al.* (2015). A PfRH5-based vaccine is efficacious against Heterologous strain blood-stage *Plasmodium falciparum* infection in aotus monkeys. Cell host & microbe *17*, 130-139.

Dreyer, A.M., Matile, H., Papastogiannidis, P., Kamber, J., Favuzza, P., Voss, T.S., Wittlin, S., and Pluschke, G. (2012). Passive immunoprotection of *Plasmodium falciparum*-infected mice designates the CyRPA as candidate malaria vaccine antigen. J Immunol *188*, 6225-6237.

Duraisingh, M., Voss, T., Marty, A., Duffy, M., Good, R., Thompson, J., Freitas-Junior, L., Scherf, A., Crabb, B., and Cowman, A. (2005). Heterochromatin silencing and locus repositioning linked to regulation of virulence genes in Plasmodium faiciparum. Cell *121*, 13-24.

Dvorin, J.D., Martyn, D.C., Patel, S.D., Grimley, J.S., Collins, C.R., Hopp, C.S., Bright, A.T., Westenberger, S., Winzeler, E., Blackman, M.J., *et al.* (2010). A plant-like kinase in Plasmodium falciparum regulates parasite egress from erythrocytes. Science *328*, 910-912.

Egan, E.S., Jiang, R.H., Moechtar, M.A., Barteneva, N.S., Weekes, M.P., Nobre, L.V., Gygi, S.P., Paulo, J.A., Frantzreb, C., Tani, Y., *et al.* (2015). Malaria. A forward genetic screen

identifies erythrocyte CD55 as essential for *Plasmodium falciparum* invasion. Science *348*, 711-714.

Epstein, J.E., Tewari, K., Lyke, K.E., Sim, B.K., Billingsley, P.F., Laurens, M.B., Gunasekera, A., Chakravarty, S., James, E.R., Sedegah, M., *et al.* (2011). Live attenuated malaria vaccine designed to protect through hepatic CD8(+) T cell immunity. Science *334*, 475-480.

Felgner, P.L., Roestenberg, M., Liang, L., Hung, C., Jain, A., Pablo, J., Nakajima-Sasaki, R., Molina, D., Teelen, K., Hermsen, C.C., *et al.* (2013). Pre-erythrocytic antibody profiles induced by controlled human malaria infections in healthy volunteers under chloroquine prophylaxis. Sci Rep *3*, 3549.

Fowkes, F.J., Richards, J.S., Simpson, J.A., and Beeson, J.G. (2010). The relationship between anti-merozoite antibodies and incidence of *Plasmodium falciparum* malaria: A systematic review and meta-analysis. PLoS Med *7*, e1000218.

Freitas-Junior, L.H., Hernandez-Rivas, R., Ralph, S.A., Montiel-Condado, D., Ruvalcaba-Salazar, O.K., Rojas-Meza, A.P., Mancio-Silva, L., Leal-Silvestre, R.J., Gontijo, A.M., Shorte, S., *et al.* (2005). Telomeric heterochromatin propagation and histone acetylation control mutually exclusive expression of antigenic variation genes in malaria parasites. Cell *121*, 25-36.

Galinski, M.R., Medina, C.C., Ingravallo, P., and Barnwell, J.W. (1992). A reticulocyte-binding protein complex of *Plasmodium vivax* merozoites. Cell *69*, 1213-1226.

Gamo, F.J., Sanz, L.M., Vidal, J., de Cozar, C., Alvarez, E., Lavandera, J.L., Vanderwall, D.E., Green, D.V., Kumar, V., Hasan, S., *et al.* (2010). Thousands of chemical starting points for antimalarial lead identification. Nature *465*, 305-310.

Gao, X., Gunalan, K., Yap, S.S., and Preiser, P.R. (2013). Triggers of key calcium signals during erythrocyte invasion by *Plasmodium falciparum*. Nat Commun *4*, 2862.

Gardner, M.J., Hall, N., Fung, E., White, O., Berriman, M., Hyman, R.W., Carlton, J.M., Pain, A., Nelson, K.E., Bowman, S., *et al.* (2002). Genome sequence of the human malaria parasite *Plasmodium falciparum*. Nature *419*, 498-511.

Gilson, P.R., and Crabb, B.S. (2009). Morphology and kinetics of the three distinct phases of red blood cell invasion by *Plasmodium falciparum* merozoites. Int J Parasitol *39*, 91-96.

Gilson, P.R., Nebl, T., Vukcevic, D., Moritz, R.L., Sargeant, T., Speed, T.P., Schofield, L., and Crabb, B.S. (2006). Identification and stoichiometry of glycosylphosphatidylinositol-anchored membrane proteins of the human malaria parasite *Plasmodium falciparum*. Molecular & cellular proteomics : MCP *5*, 1286-1299.

Giovannini, D., Spath, S., Lacroix, C., Perazzi, A., Bargieri, D., Lagal, V., Lebugle, C., Combe, A., Thiberge, S., Baldacci, P., *et al.* (2011). Independent roles of apical membrane antigen 1 and rhoptry neck proteins during host cell invasion by apicomplexa. Cell host & microbe *10*, 591-602.

Glushakova, S., Yin, D., Li, T., and Zimmerberg, J. (2005). Membrane transformation during malaria parasite release from human red blood cells. Curr Biol *15*, 1645-1650.

Goel, V.K., Li, X., Chen, H., Liu, S.C., Chishti, A.H., and Oh, S.S. (2003). Band 3 is a host receptor binding merozoite surface protein 1 during the *Plasmodium falciparum* invasion of erythrocytes. Proc Natl Acad Sci U S A *100*, 5164-5169.

Gomez-Perez, G.P., van Bruggen, R., Grobusch, M.P., and Dobano, C. (2014). *Plasmodium falciparum* malaria and invasive bacterial co-infection in young African children: the dysfunctional spleen hypothesis. Malaria journal *13*, 335.

Good, M.F., Reiman, J.M., Rodriguez, I.B., Ito, K., Yanow, S.K., El-Deeb, I.M., Batzloff, M.R., Stanisic, D.I., Engwerda, C., Spithill, T., *et al.* (2013). Cross-species malaria immunity induced by chemically attenuated parasites. J Clin Invest.

Greenwood, B., and Doumbo, O.K. (2016). Implementation of the malaria candidate vaccine RTS,S/AS01. Lancet *387*, 318-319.

Gruring, C., Heiber, A., Kruse, F., Flemming, S., Franci, G., Colombo, S.F., Fasana, E., Schoeler, H., Borgese, N., Stunnenberg, H.G., *et al.* (2012). Uncovering common principles in protein export of malaria parasites. Cell host & microbe *12*, 717-729.

Guerin, P.J., Bates, S.J., and Sibley, C.H. (2009). Global resistance surveillance: ensuring antimalarial efficacy in the future. Curr Opin Infect Dis *22*, 593-600.

Hanson, J., Lee, S.J., Hossain, M.A., Anstey, N.M., Charunwatthana, P., Maude, R.J., Kingston, H.W., Mishra, S.K., Mohanty, S., Plewes, K., *et al.* (2015). Microvascular obstruction and endothelial activation are independently associated with the clinical manifestations of severe falciparum malaria in adults: an observational study. BMC Med *13*, 122.

Haynes, J.D., Dalton, J.P., Klotz, F.W., McGinniss, M.H., Hadley, T.J., Hudson, D.E., and Miller, L.H. (1988). Receptor-like specificity of a *Plasmodium knowlesi* malarial protein that binds to duffy antigen ligands on erythrocytes. J Exp Med *167*, 1873-1881.

Haynes, R.K., Cheu, K.W., Chan, H.W., Wong, H.N., Li, K.Y., Tang, M.M., Chen, M.J., Guo, Z.F., Guo, Z.H., Sinniah, K., *et al.* (2012). Interactions between artemisinins and other antimalarial drugs in relation to the cofactor model--a unifying proposal for drug action. ChemMedChem *7*, 2204-2226.

Hayton, K., Gaur, D., Liu, A., Takahashi, J., Henschen, B., Singh, S., Lambert, L., Furuya, T., Bouttenot, R., Doll, M., *et al.* (2008). Erythrocyte binding protein PfRH5 polymorphisms determine species-specific pathways of *Plasmodium falciparum* invasion. Cell host & microbe *4*, 40-51.

Hellmann, J.K., Munter, S., Kudryashev, M., Schulz, S., Heiss, K., Muller, A.K., Matuschewski, K., Spatz, J.P., Schwarz, U.S., and Frischknecht, F. (2011). Environmental constraints guide migration of malaria parasites during transmission. PLoS Pathog *7*, e1002080.

Hendriksen, I.C., White, L.J., Veenemans, J., Mtove, G., Woodrow, C., Amos, B., Saiwaew, S., Gesase, S., Nadjm, B., Silamut, K., *et al.* (2013). Defining falciparum-malaria-attributable severe febrile illness in moderate-to-high transmission settings on the basis of plasma PfHRP2 concentration. J Infect Dis *207*, 351-361.

Herrera, R., Anderson, C., Kumar, K., Molina-Cruz, A., Nguyen, V., Burkhardt, M., Reiter, K., Shimp, R., Jr., Howard, R.F., Srinivasan, P., *et al.* (2015). Reversible Conformational Change in the *Plasmodium falciparum* Circumsporozoite Protein Masks Its Adhesion Domains. Infect Immun *83*, 3771-3780.

Hill, A.V. (2011). Vaccines against malaria. Philos Trans R Soc Lond B Biol Sci *366*, 2806-2814.

Hiller, N.L., Bhattacharjee, S., van Ooij, C., Liolios, K., Harrison, T., Lopez-Estrano, C., and Haldar, K. (2004). A host-targeting signal in virulence proteins reveals a secretome in malarial infection. Science *306*, 1934-1937.

Holder, A.A. (1994). Proteins on the surface of the malaria parasite and cell invasion. Parasitology *108*, S5-S18.

Hviid, L., and Jensen, A.T. (2015). PfEMP1 - A Parasite Protein Family of Key Importance in Plasmodium falciparum Malaria Immunity and Pathogenesis. Adv Parasitol *88*, 51-84.

Ishino, T., Chinzei, Y., and Yuda, M. (2005). A *Plasmodium* sporozoite protein with a membrane attack complex domain is required for breaching the liver sinusoidal cell layer prior to hepatocyte infection. Cell Microbiol *7*, 199-208.

Ishino, T., Yano, K., Chinzei, Y., and Yuda, M. (2004). Cell-passage activity is required for the malarial parasite to cross the liver sinusoidal cell layer. PLoS Biol *2*, E4.

Jimenez-Diaz, M.B., Ebert, D., Salinas, Y., Pradhan, A., Lehane, A.M., Myrand-Lapierre, M.E., O'Loughlin, K.G., Shackleford, D.M., Justino de Almeida, M., Carrillo, A.K., *et al.* (2014). (+)-SJ733, a clinical candidate for malaria that acts through ATP4 to induce rapid host-

mediated clearance of Plasmodium. Proceedings of the National Academy of Sciences of the United States of America *111*, E5455-5462.

Kabyemela, E., Goncalves, B.P., Prevots, D.R., Morrison, R., Harrington, W., Gwamaka, M., Kurtis, J.D., Fried, M., and Duffy, P.E. (2013). Cytokine profiles at birth predict malaria severity during infancy. PloS one *8*, e77214.

Kaiser, K., Camargo, N., Coppens, I., Morrisey, J.M., Vaidya, A.B., and Kappe, S.H. (2004). A member of a conserved *Plasmodium* protein family with membrane-attack complex/perforin (MACPF)-like domains localizes to the micronemes of sporozoites. Mol Biochem Parasitol *133*, 15-26.

Kariu, T., Ishino, T., Yano, K., Chinzei, Y., and Yuda, M. (2006). CelTOS, a novel malarial protein that mediates transmission to mosquito and vertebrate hosts. Mol Microbiol *59*, 1369-1379.

Kaushansky, A., Douglass, A.N., Arang, N., Vigdorovich, V., Dambrauskas, N., Kain, H.S., Austin, L.S., Sather, D.N., and Kappe, S.H. (2015). Malaria parasites target the hepatocyte receptor EphA2 for successful host infection. Science *350*, 1089-1092.

Kennedy, A.T., Schmidt, C.Q., Thompson, J.K., Weiss, G.E., Taechalertpaisarn, T., Gilson, P.R., Barlow, P.N., Crabb, B.S., Cowman, A.F., and Tham, W.H. (2016). Recruitment of Factor H as a Novel Complement Evasion Strategy for Blood-Stage *Plasmodium falciparum* Infection. J Immunol *196*, 1239-1248.

Khunrae, P., Dahlback, M., Nielsen, M.A., Andersen, G., Ditlev, S.B., Resende, M., Pinto, V.V., Theander, T.G., Higgins, M.K., and Salanti, A. (2010). Full-length recombinant *Plasmodium falciparum* VAR2CSA binds specifically to CSPG and induces potent parasite adhesion-blocking antibodies. J Mol Biol *397*, 826-834.

Kleinschmidt, I., Schwabe, C., Benavente, L., Torrez, M., Ridl, F.C., Segura, J.L., Ehmer, P., and Nchama, G.N. (2009). Marked increase in child survival after four years of intensive malaria control. Am J Trop Med Hyg *80*, 882-888.

Klonis, N., Crespo-Ortiz, M.P., Bottova, I., Abu-Bakar, N., Kenny, S., Rosenthal, P.J., and Tilley, L. (2011). Artemisinin activity against *Plasmodium falciparum* requires hemoglobin uptake and digestion. Proceedings of the National Academy of Sciences of the United States of America *108*, 11405-11410.

Kojin, B.B., Costa-da-Silva, A.L., Maciel, C., Henriques, D.A., Carvalho, D.O., Martin, K., Marinotti, O., James, A.A., Bonaldo, M.C., and Capurro, M.L. (2016). Endogenously-expressed NH2-terminus of circumsporozoite protein interferes with sporozoite invasion of mosquito salivary glands. Malar J *15*, 153.

Kokkonda, S., Deng, X., White, K.L., Coteron, J.M., Marco, M., de Las Heras, L., White, J., El Mazouni, F., Tomchick, D.R., Manjalanagara, K., *et al.* (2016). Tetrahydro-2-naphthyl and 2-indanyl triazolopyrimidines targeting *Plasmodium falciparum* dihydroorotate dehydrogenase display potent and selective antimalarial activity. J Med Chem.

Kuhen, K.L., Chatterjee, A.K., Rottmann, M., Gagaring, K., Borboa, R., Buenviaje, J., Chen, Z., Francek, C., Wu, T., Nagle, A., *et al.* (2014). KAF156 is an antimalarial clinical candidate with potential for use in prophylaxis, treatment, and prevention of disease transmission. Antimicrob Agents Chemother *58*, 5060-5067.

Kulzer, S., Rug, M., Brinkmann, K., Cannon, P., Cowman, A., Lingelbach, K., Blatch, G.L., Maier, A.G., and Przyborski, J.M. (2010). Parasite-encoded Hsp40 proteins define novel mobile structures in the cytosol of the *P. falciparum*-infected erythrocyte. Cell Microbiol *12*, 1398-1420.

Kwiatkowski, D., Hill, A.V.S., Sambou, I., Twumasi, P., Castracane, J., Manogue, K.R., Cerami, A., Brewster, D.R., and Greenwood, B.M. (1990). TNF concentration in fatal cerebral, non-fatal cerebral, and uncomplicated *Plasmodium falciparum* malaria. Lancet *336*, 1201-1204.

Lacerda, M.V., Fragoso, S.C., Alecrim, M.G., Alexandre, M.A., Magalhaes, B.M., Siqueira, A.M., Ferreira, L.C., Araujo, J.R., Mourao, M.P., Ferrer, M., *et al.* (2012). Postmortem characterization of patients with clinical diagnosis of *Plasmodium vivax* malaria: to what extent does this parasite kill? Clin Infect Dis *55*, e67-74.

Lamarque, M.H., Roques, M., Kong-Hap, M., Tonkin, M.L., Rugarabamu, G., Marq, J.B., Penarete-Vargas, D.M., Boulanger, M.J., Soldati-Favre, D., and Lebrun, M. (2014). Plasticity and redundancy among AMA-RON pairs ensure host cell entry of *Toxoplasma* parasites. Nat Commun *5*, 4098.

Langhorne, J., Ndungu, F.M., Sponaas, A.M., and Marsh, K. (2008). Immunity to malaria: more questions than answers. Nat Immunol *9*, 725-732.

Leong, F.J., Li, R., Jain, J.P., Lefevre, G., Magnusson, B., Diagana, T.T., and Pertel, P. (2014). A first-in-human randomized, double-blind, placebo-controlled, single- and multiple-ascending oral dose study of novel antimalarial Spiroindolone KAE609 (Cipargamin) to assess its safety, tolerability, and pharmacokinetics in healthy adult volunteers. Antimicrob Agents Chemother *58*, 6209-6214.

Lin, C.S., Uboldi, A.D., Epp, C., Bujard, H., Tsuboi, T., Czabotar, P.E., and Cowman, A.F. (2016). Multiple *P. falciparum* Merozoite Surface Protein 1 Complexes Mediate Merozoite Binding to Human Erythrocytes. J Biol Chem.

Lin, C.S., Uboldi, A.D., Marapana, D., Czabotar, P.E., Epp, C., Bujard, H., Taylor, N.L., Perugini, M.A., Hodder, A.N., and Cowman, A.F. (2014). The merozoite surface protein 1 complex is a platform for binding to human erythrocytes by *Plasmodium falciparum*. J Biol Chem *289*, 25655-28669.

Llanos-Cuentas, A., Lacerda, M.V., Rueangweerayut, R., Krudsood, S., Gupta, S.K., Kochar, S.K., Arthur, P., Chuenchom, N., Mohrle, J.J., Duparc, S., *et al.* (2014). Tafenoquine plus chloroquine for the treatment and relapse prevention of *Plasmodium vivax* malaria (DETECTIVE): a multicentre, double-blind, randomised, phase 2b dose-selection study. Lancet *383*, 1049-1058.

Lopaticki, S., Maier, A.G., Thompson, J., Wilson, D.W., Tham, W.H., Triglia, T., Gout, A., Speed, T.P., Beeson, J.G., Healer, J., *et al.* (2011). Reticulocyte and erythrocyte binding-like proteins function cooperatively in invasion of human erythrocytes by malaria parasites. Infect Immun *79*, 1107-1117.

Luxemburger, C., Ricci, F., Nosten, F., Raimond, D., Bathet, S., and White, N.J. (1997). The epidemiology of severe malaria in an area of low transmission in Thailand. Trans R Soc Trop Med Hyg *91*, 256-262.

Maier, A., Duraisingh, M., Reeder, J., Patel, S., Kazura, J., Zimmerman, P., and Cowman, A. (2003). Plasmodium falciparum erythrocyte invasion through glycophorin C and selection for Gerbich negativity in human populations. Nature Medicine *9*, 87-92.

March, S., Ng, S., Velmurugan, S., Galstian, A., Shan, J., Logan, D.J., Carpenter, A.E., Thomas, D., Sim, B.K., Mota, M.M., *et al.* (2013). A microscale human liver platform that supports the hepatic stages of Plasmodium falciparum and vivax. Cell host & microbe *14*, 104-115.

Marsh, K., Forster, D., Waruiru, C., Mwangi, I., Winstanley, M., Marsh, V., Newton, C., Winstanley, P., Warn, P., Peshu, N., *et al.* (1995). Indicators of life-threatening malaria in African children. N Engl J Med *332*, 1399-1404.

Marti, M., Good, R., Rug, M., Knuepfer, E., and Cowman, A. (2004). Targeting malaria virulence and remodeling proteins to the host erythrocyte. Science *306*, 1930-1933.

Mayer, D.C., Cofie, J., Jiang, L., Hartl, D.L., Tracy, E., Kabat, J., Mendoza, L.H., and Miller, L.H. (2009). Glycophorin B is the erythrocyte receptor of *Plasmodium falciparum* erythrocyte-binding ligand, EBL-1. Proc Natl Acad Sci USA *106*, 5348-5352.

Mayer, D.C., Jiang, L., Achur, R.N., Kakizaki, I., Gowda, D.C., and Miller, L.H. (2006). The glycophorin C N-linked glycan is a critical component of the ligand for the *Plasmodium falciparum* erythrocyte receptor BAEBL. Proc Natl Acad Sci U S A *103*, 2358-2362.

Mbengue, A., Bhattacharjee, S., Pandharkar, T., Liu, H., Estiu, G., Stahelin, R.V., Rizk, S.S., Njimoh, D.L., Ryan, Y., Chotivanich, K., *et al.* (2015). A molecular mechanism of artemisinin resistance in *Plasmodium falciparum* malaria. Nature *520*, 683-687.

McLean, A.R., Ataide, R., Simpson, J.A., Beeson, J.G., and Fowkes, F.J. (2015). Malaria and immunity during pregnancy and postpartum: a tale of two species. Parasitology *142*, 999-1015.

McMillan, P.J., Millet, C., Batinovic, S., Maiorca, M., Hanssen, E., Kenny, S., Muhle, R.A., Melcher, M., Fidock, D.A., Smith, J.D., *et al.* (2013). Spatial and temporal mapping of the PfEMP1 export pathway in *Plasmodium falciparum*. Cell Microbiol *15*, 1401-1418.

McNamara, C.W., Lee, M.C., Lim, C.S., Lim, S.H., Roland, J., Nagle, A., Simon, O., Yeung, B.K., Chatterjee, A.K., McCormack, S.L., *et al.* (2013). Targeting *Plasmodium* PI(4)K to eliminate malaria. Nature *504*, 248-253.

Menard, S., Ben Haddou, T., Ramadani, A.P., Ariey, F., Iriart, X., Beghain, J., Bouchier, C., Witkowski, B., Berry, A., Mercereau-Puijalon, O., *et al.* (2015). Induction of Multidrug Tolerance in *Plasmodium falciparum* by Extended Artemisinin Pressure. Emerg Infect Dis *21*, 1733-1741.

Mikolajczak, S.A., Lakshmanan, V., Fishbaugher, M., Camargo, N., Harupa, A., Kaushansky, A., Douglass, A.N., Baldwin, M., Healer, J., O'Neill, M., *et al.* (2014). A next-generation genetically attenuated *Plasmodium falciparum* parasite created by triple gene deletion. Mol Ther *22*, 1707-1715.

Mikolajczak, S.A., Vaughan, A.M., Kangwanrangsan, N., Roobsoong, W., Fishbaugher, M., Yimamnuaychok, N., Rezakhani, N., Lakshmanan, V., Singh, N., Kaushansky, A., *et al.* (2015). *Plasmodium vivax* liver stage development and hypnozoite persistence in human liver-chimeric mice. Cell host & microbe *17*, 526-535.

Miotto, O., Amato, R., Ashley, E.A., MacInnis, B., Almagro-Garcia, J., Amaratunga, C., Lim, P., Mead, D., Oyola, S.O., Dhorda, M., *et al.* (2015). Genetic architecture of artemisinin-resistant *Plasmodium falciparum*. Nat Genet *47*, 226-234.

Mok, S., Ashley, E.A., Ferreira, P.E., Zhu, L., Lin, Z., Yeo, T., Chotivanich, K., Imwong, M., Pukrittayakamee, S., Dhorda, M., *et al.* (2015). Drug resistance. Population transcriptomics of human malaria parasites reveals the mechanism of artemisinin resistance. Science *347*, 431-435.

Moreira, C.K., Templeton, T.J., Lavazec, C., Hayward, R.E., Hobbs, C.V., Kroeze, H., Janse, C.J., Waters, A.P., Sinnis, P., and Coppi, A. (2008). The *Plasmodium* TRAP/MIC2 family member, TRAP-Like Protein (TLP), is involved in tissue traversal by sporozoites. Cell Microbiol *10*, 1505-1516.

Mota, M.M., Hafalla, J.C., and Rodriguez, A. (2002). Migration through host cells activates *Plasmodium* sporozoites for infection. Nat Med *8*, 1318-1322.

Moxon, C.A., Wassmer, S.C., Milner, D.A., Jr., Chisala, N.V., Taylor, T.E., Seydel, K.B., Molyneux, M.E., Faragher, B., Esmon, C.T., Downey, C., *et al.* (2013). Loss of endothelial protein C receptors links coagulation and inflammation to parasite sequestration in cerebral malaria in African children. Blood *122*, 842-851.

Naing, C., Whittaker, M.A., Nyunt Wai, V., and Mak, J.W. (2014). Is *Plasmodium vivax* malaria a severe malaria?: a systematic review and meta-analysis. PLoS Negl Trop Dis *8*, e3071.

Naka, I., Patarapotikul, J., Hananantachai, H., Imai, H., and Ohashi, J. (2014). Association of the endothelial protein C receptor (PROCR) rs867186-G allele with protection from severe malaria. Malaria journal *13*, 105.

Ndungu, F.M., Olotu, A., Mwacharo, J., Nyonda, M., Apfeld, J., Mramba, L.K., Fegan, G.W., Bejon, P., and Marsh, K. (2012). Memory B cells are a more reliable archive for historical antimalarial responses than plasma antibodies in no-longer exposed children. Proceedings of the National Academy of Sciences of the United States of America *109*, 8247-8252.

Nikolaeva, D., Draper, S.J., and Biswas, S. (2015). Toward the development of effective transmission-blocking vaccines for malaria. Expert Rev Vaccines *14*, 653-660.

Noedl, H., Se, Y., Schaecher, K., Smith, B.L., Socheat, D., Fukuda, M.M., and Artemisinin Resistance in Cambodia 1 Study, C. (2008). Evidence of artemisinin-resistant malaria in western Cambodia. N Engl J Med *359*, 2619-2620.

Nunes-Silva, S., Dechavanne, S., Moussiliou, A., Pstrag, N., Semblat, J.P., Gangnard, S., Tuikue-Ndam, N., Deloron, P., Chene, A., and Gamain, B. (2015). Beninese children with cerebral malaria do not develop humoral immunity against the IT4-VAR19-DC8 PfEMP1 variant linked to EPCR and brain endothelial binding. Malaria journal *14*, 493.

O'Meara, W.P., Bejon, P., Mwangi, T.W., Okiro, E.A., Peshu, N., Snow, R.W., Newton, C.R., and Marsh, K. (2008). Effect of a fall in malaria transmission on morbidity and mortality in Kilifi, Kenya. Lancet *372*, 1555-1562.

Ochola, L.B., Siddondo, B.R., Ocholla, H., Nkya, S., Kimani, E.N., Williams, T.N., Makale, J.O., Liljander, A., Urban, B.C., Bull, P.C., *et al.* (2011). Specific receptor usage in Plasmodium falciparum cytoadherence is associated with disease outcome. PloS one *6*, e14741.

Orlandi, P.A., Klotz, F.W., and Haynes, J.D. (1992). A malaria invasion receptor, the 175-kilodalton erythrocyte binding antigen of *Plasmodium falciparum* recognizes the terminal Neu5Ac(alpha2-3)Gal- sequences of glycophorin A. J Cell Biol *116*, 901-909.

Osier, F.H., Mackinnon, M.J., Crosnier, C., Fegan, G., Kamuyu, G., Wanaguru, M., Ogada, E., McDade, B., Rayner, J.C., Wright, G.J., *et al.* (2014). New antigens for a multicomponent blood-stage malaria vaccine. Sci Transl Med *6*, 247ra102.

Ozaki, L.S., Svec, P., Nussenzweig, R.S., Nussenzweig, V., and Godson, G.N. (1983). Structure of the *Plasmodium knowlesi* gene coding for the circumsporozoite protein. Cell *34*, 815-822.

Pachlatko, E., Rusch, S., Muller, A., Hemphill, A., Tilley, L., Hanssen, E., and Beck, H.P. (2010). MAHRP2, an exported protein of *Plasmodium falciparum*, is an essential component of Maurer's cleft tethers. Mol Microbiol *77*, 1136-1152.

Paul, A.S., Saha, S., Engelberg, K., Jiang, R.H., Coleman, B.I., Kosber, A.L., Chen, C.T., Ganter, M., Espy, N., Gilberger, T.W., *et al.* (2015). Parasite Calcineurin Regulates Host Cell Recognition and Attachment by Apicomplexans. Cell host & microbe *18*, 49-60.

Payne, R.O., Milne, K.H., Elias, S.C., Edwards, N.J., Douglas, A.D., Brown, R.E., Silk, S.E., Biswas, S., Miura, K., Roberts, R., *et al.* (2016). Demonstration of the Blood-Stage *Plasmodium falciparum* Controlled Human Malaria Infection Model to Assess Efficacy of the *P. falciparum* Apical Membrane Antigen 1 Vaccine, FMP2.1/AS01. J Infect Dis.

Peterson, M.G., Marshall, V.M., Smythe, J.A., Crewther, P.E., Lew, A., Silva, A., Anders, R.F., and Kemp, D.J. (1989). Integral membrane protein located in the apical complex of *Plasmodium falciparum*. Mol Cell Biol *9*, 3151-3154.

Popovici, J., Kao, S., Eal, L., Bin, S., Kim, S., and Menard, D. (2015). Reduced polymorphism in the Kelch propeller domain in *Plasmodium vivax* isolates from Cambodia. Antimicrob Agents Chemother *59*, 730-733.

Poukchanski, A., Fritz, H.M., Tonkin, M.L., Treeck, M., Boulanger, M.J., and Boothroyd, J.C. (2013). Toxoplasma gondii sporozoites invade host cells using two novel paralogues of RON2 and AMA1. PloS one *8*, e70637.

Rahimi, B.A., Thakkinstian, A., White, N.J., Sirivichayakul, C., Dondorp, A.M., and Chokejindachai, W. (2014). Severe vivax malaria: a systematic review and meta-analysis of clinical studies since 1900. Malaria journal *13*, 481.

Rayner, J.C., Galinski, M.R., Ingravallo, P., and Barnwell, J.W. (2000). Two *Plasmodium falciparum* genes express merozoite proteins that are related to *Plasmodium vivax* and *Plasmodium yoelii* adhesive proteins involved in host cell selection and invasion. Proc Natl Acad Sci USA *97*, 9648-9653.

Reddy, K.S., Amlabu, E., Pandey, A.K., Mitra, P., Chauhan, V.S., and Gaur, D. (2015). Multiprotein complex between the GPI-anchored CyRPA with PfRH5 and PfRipr is crucial for *Plasmodium falciparum* erythrocyte invasion. Proc Natl Acad Sci U S A.

Reyes-Sandoval, A., Rollier, C.S., Milicic, A., Bauza, K., Cottingham, M.G., Tang, C.K., Dicks, M.D., Wang, D., Longley, R.J., Wyllie, D.H., *et al.* (2012). Mixed vector immunization with recombinant adenovirus and MVA can improve vaccine efficacy while decreasing antivector immunity. Mol Ther *20*, 1633-1647.

Richard, D., MacRaild, C.A., Riglar, D.T., Chan, J.A., Foley, M., Baum, J., Ralph, S.A., Norton, R.S., and Cowman, A.F. (2010). Interaction between *Plasmodium falciparum* apical membrane antigen 1 and the rhoptry neck protein complex defines a key step in the erythrocyte invasion process of malaria parasites. J Biol Chem *285*, 14815-14822.

Richards, J.S., Arumugam, T.U., Reiling, L., Healer, J., Hodder, A.N., Fowkes, F.J., Cross, N., Langer, C., Takeo, S., Uboldi, A.D., *et al.* (2013). Identification and Prioritization of Merozoite Antigens as Targets of Protective Human Immunity to *Plasmodium falciparum* Malaria for Vaccine and Biomarker Development. J Immunol *191*, 795-809.

Riglar, D.T., Richard, D., Wilson, D.W., Boyle, M.J., Dekiwadia, C., Turnbull, L., Angrisano, F., Marapana, D.S., Rogers, K.L., Whitchurch, C.B., *et al.* (2011). Super-resolution dissection of coordinated events during malaria parasite invasion of the human erythrocyte. Cell host & microbe *9*, 9-20.

Riley, E.M., and Stewart, V.A. (2013). Immune mechanisms in malaria: new insights in vaccine development. Nat Med *19*, 168-178.

Risco-Castillo, V., Topcu, S., Marinach, C., Manzoni, G., Bigorgne, A.E., Briquet, S., Baudin, X., Lebrun, M., Dubremetz, J.F., and Silvie, O. (2015). Malaria Sporozoites Traverse Host Cells within Transient Vacuoles. Cell host & microbe *18*, 593-603.

Robson, K.J., Frevert, U., Reckmann, I., Cowan, G., Beier, J., Scragg, I.G., Takehara, K., Bishop, D.H., Pradel, G., Sinden, R., *et al.* (1995). Thrombospondin-related adhesive protein (TRAP) of Plasmodium falciparum: expression during sporozoite ontogeny and binding to human hepatocytes. EMBO J *14*, 3883-3894.

Robson, K.J., Hall, J.R., Jennings, M.W., Harris, T.J., Marsh, K., Newbold, C.I., Tate, V.E., and Weatherall, D.J. (1988). A highly conserved amino-acid sequence in thrombospondin, properdin and in proteins from sporozoites and blood stages of a human malaria parasite. Nature *335*, 79-82.

Rodrigues, C.D., Hannus, M., Prudencio, M., Martin, C., Goncalves, L.A., Portugal, S., Epiphanio, S., Akinc, A., Hadwiger, P., Jahn-Hofmann, K., *et al.* (2008). Host scavenger receptor SR-BI plays a dual role in the establishment of malaria parasite liver infection. Cell host & microbe *4*, 271-282.

Roestenberg, M., McCall, M., Hopman, J., Wiersma, J., Luty, A.J., van Gemert, G.J., van de Vegte-Bolmer, M., van Schaijk, B., Teelen, K., Arens, T., *et al.* (2009). Protection against a malaria challenge by sporozoite inoculation. N Engl J Med *361*, 468-477.

Rottmann, M., McNamara, C., Yeung, B.K., Lee, M.C., Zou, B., Russell, B., Seitz, P., Plouffe, D.M., Dharia, N.V., Tan, J., *et al.* (2010). Spiroindolones, a potent compound class for the treatment of malaria. Science *329*, 1175-1180.

Saenz, F.E., Balu, B., Smith, J., Mendonca, S.R., and Adams, J.H. (2008). The transmembrane isoform of *Plasmodium falciparum* MAEBL is essential for the invasion of *Anopheles* salivary glands. PloS one *3*, e2287.

Sargeant, T., Marti, M., Caler, E., Carlton, J., Simpson, K., Speed, T., and Cowman, A. (2006). Lineage-specific expansion of proteins exported to erythrocytes in malaria parasites. Genome Biology *7*.

Scherf, A., Hernandez-Rivas, R., Buffet, P., Bottius, E., Benatar, C., Pouvelle, B., Gysin, J., and Lanzer, M. (1998). Antigenic variation in malaria: in situ switching, relaxed and mutally exclusive transcription of var genes during intra-erythrocytic development in Plasmodium falciparum. EMBO J *17*, 5418-5426.

Scott, J.A., Berkley, J.A., Mwangi, I., Ochola, L., Uyoga, S., Macharia, A., Ndila, C., Lowe, B.S., Mwarumba, S., Bauni, E., *et al.* (2011). Relation between falciparum malaria and bacteraemia in Kenyan children: a population-based, case-control study and a longitudinal study. Lancet *378*, 1316-1323.

Seder, R.A., Chang, L.J., Enama, M.E., Zephir, K.L., Sarwar, U.N., Gordon, I.J., Holman, L.A., James, E.R., Billingsley, P.F., Gunasekera, A., *et al.* (2013). Protection against malaria by intravenous immunization with a nonreplicating sporozoite vaccine. Science *341*, 1359-1365.

Shen, B., and Sibley, L.D. (2014). *Toxoplasma* aldolase is required for metabolism but dispensable for host-cell invasion. Proceedings of the National Academy of Sciences of the United States of America *111*, 3567-3572.

Silmon de Monerri, N.C., Flynn, H.R., Campos, M.G., Hackett, F., Koussis, K., Withers-Martinez, C., Skehel, J.M., and Blackman, M.J. (2011). Global identification of multiple substrates for *Plasmodium falciparum* SUB1, an essential malarial processing protease. Infect Immun *79*, 1086-1097.

Silvie, O., Franetich, J.F., Charrin, S., Mueller, M.S., Siau, A., Bodescot, M., Rubinstein, E., Hannoun, L., Charoenvit, Y., Kocken, C.H., *et al.* (2004). A role for apical membrane antigen 1 during invasion of hepatocytes by *Plasmodium falciparum* sporozoites. The Journal of biological chemistry *279*, 9490-9496.

Silvie, O., Rubinstein, E., Franetich, J.F., Prenant, M., Belnoue, E., Renia, L., Hannoun, L., Eling, W., Levy, S., Boucheix, C., *et al.* (2003). Hepatocyte CD81 is required for *Plasmodium falciparum* and *Plasmodium yoelii* sporozoite infectivity. Nat Med *9*, 93-96.

Singh, S., Alam, M.M., Pal-Bhowmick, I., Brzostowski, J.A., and Chitnis, C.E. (2010). Distinct external signals trigger sequential release of apical organelles during erythrocyte invasion by malaria parasites. PLoS Pathog *6*, e1000746.

Slater, H.C., Griffin, J.T., Ghani, A.C., and Okell, L.C. (2016). Assessing the potential impact of artemisinin and partner drug resistance in sub-Saharan Africa. Malaria journal *15*, 10.

Smith, J.D., Chitnis, C.E., Craig, A.G., Roberts, D.J., Hudson-Taylor, D.E., Peterson, D.S., Pinches, R., Newbold, C.I., and Miller, L.H. (1995). Switches in expression of *Plasmodium falciparum var* genes correlate with changes in antigenic and cytoadherent phenotypes of infected erythrocytes. Cell *82*, 101-110.

Smith, J.D., Rowe, J.A., Higgins, M.K., and Lavstsen, T. (2013). Malaria's deadly grip: cytoadhesion of *Plasmodium falciparum*-infected erythrocytes. Cell Microbiol *15*, 1976-1983.

Snow, R.W., Omunbo, J.A., Lowe, B., Molyneux, C.S., Obiero, J.O., Palmer, A., Weber, M.W., Pinder, M., Nahlen, B., Obonyo, C., *et al.* (1997). Relation between severe malaria morbidity in children and level of Plasmodium falciparum transmission in Africa. Lancet *349*, 1650-1654.

Spangenberg, T., Burrows, J.N., Kowalczyk, P., McDonald, S., Wells, T.N., and Willis, P. (2013). The open access malaria box: a drug discovery catalyst for neglected diseases. PloS one *8*, e62906.

Spillman, N.J., Allen, R.J., McNamara, C.W., Yeung, B.K., Winzeler, E.A., Diagana, T.T., and Kirk, K. (2013). Na(+) regulation in the malaria parasite *Plasmodium falciparum* involves the cation ATPase PfATP4 and is a target of the spiroindolone antimalarials. Cell host & microbe *13*, 227-237.

Spring, M., Murphy, J., Nielsen, R., Dowler, M., Bennett, J.W., Zarling, S., Williams, J., de la Vega, P., Ware, L., Komisar, J., *et al.* (2013). First-in-human evaluation of genetically attenuated *Plasmodium falciparum* sporozoites administered by bite of Anopheles mosquitoes to adult volunteers. Vaccine *31*, 4975-4983.

Spring, M.D., Cummings, J.F., Ockenhouse, C.F., Dutta, S., Reidler, R., Angov, E., Bergmann-Leitner, E., Stewart, V.A., Bittner, S., Juompan, L., *et al.* (2009). Phase 1/2a study of the malaria vaccine candidate apical membrane antigen-1 (AMA-1) administered in adjuvant system AS01B or AS02A. PloS one *4*, e5254.

Spycher, C., Rug, M., Klonis, N., Ferguson, D., Cowman, A., Beck, H., and Tilley, L. (2006). Genesis of and trafficking to the Maurer's clefts of *Plasmodium falciparum*-infected erythrocytes. Molecular and Cellular Biology *26*, 4074-4085.

Stein, D.S., Jain, J.P., Kangas, M., Lefevre, G., Machineni, S., Griffin, P., and Lickliter, J. (2015). Open-label, single-dose, parallel-group study in healthy volunteers to determine the drug-drug interaction potential between KAE609 (cipargamin) and piperaquine. Antimicrob Agents Chemother *59*, 3493-3500.

Steinbuechel, M., and Matuschewski, K. (2009). Role for the *Plasmodium* sporozoite-specific transmembrane protein S6 in parasite motility and efficient malaria transmission. Cellular microbiology *11*, 279-288.

Straimer, J., Gnadig, N.F., Witkowski, B., Amaratunga, C., Duru, V., Ramadani, A.P., Dacheux, M., Khim, N., Zhang, L., Lam, S., *et al.* (2015). Drug resistance. K13-propeller mutations confer artemisinin resistance in *Plasmodium falciparum* clinical isolates. Science *347*, 428-431.

Sturm, A., Amino, R., van de Sand, C., Regen, T., Retzlaff, S., Rennenberg, A., Krueger, A., Pollok, J.M., Menard, R., and Heussler, V.T. (2006). Manipulation of host hepatocytes by the malaria parasite for delivery into liver sinusoids. Science *313*, 1287-1290.

Takala-Harrison, S., Jacob, C.G., Arze, C., Cummings, M.P., Silva, J.C., Dondorp, A.M., Fukuda, M.M., Hien, T.T., Mayxay, M., Noedl, H., *et al.* (2015). Independent emergence of artemisinin resistance mutations among *Plasmodium falciparum* in Southeast Asia. J Infect Dis *211*, 670-679.

Talman, A.M., Lacroix, C., Marques, S.R., Blagborough, A.M., Carzaniga, R., Menard, R., and Sinden, R.E. (2011). PbGEST mediates malaria transmission to both mosquito and vertebrate host. Mol Microbiol *82*, 462-474.

Tavares, J., Formaglio, P., Thiberge, S., Mordelet, E., Van Rooijen, N., Medvinsky, A., Menard, R., and Amino, R. (2013). Role of host cell traversal by the malaria sporozoite during liver infection. J Exp Med *210*, 905-915.

Taylor, T.E., and Molyneux, M.E. (2015). The pathogenesis of pediatric cerebral malaria: eye exams, autopsies, and neuroimaging. Ann NY Acad Sci *1342*, 44-52.

Tham, W.-H., Wilson, D.W., Lopaticki, S., Schmidt, C.Q., Tetteh-Quarcoo, P.B., Barlow, P.N., Richard, D., Corbin, J.E., Beeson, J.G., and Cowman, A.F. (2010). Complement receptor 1 is the host erythrocyte receptor for Plasmodium falciparum PfRh4 invasion ligand. Proceedings of the National Academy of Sciences of the United States of America *107*, 17327-17332.

Tham, W.H., Lim, N.T., Weiss, G.E., Lopaticki, S., Ansell, B.R., Bird, M., Lucet, I., Dorin-Semblat, D., Doerig, C., Gilson, P.R., *et al.* (2015). *Plasmodium falciparum* Adhesins Play an Essential Role in Signalling and Activation of Invasion into Human Erythrocytes. PLoS Pathog *11*, e1005343.

Theisen, M., Roeffen, W., Singh, S.K., Andersen, G., Amoah, L., van de Vegte-Bolmer, M., Arens, T., Tiendrebeogo, R.W., Jones, S., Bousema, T., *et al.* (2014). A multi-stage malaria vaccine candidate targeting both transmission and asexual parasite life-cycle stages. Vaccine *32*, 2623-2630.

Triglia, T., Thompson, J., Caruana, S., Delorenzi, M., Speed, T., and Cowman, A. (2001). Identification of proteins from *Plasmodium falciparum* that are homologous to reticulocyte binding proteins in *Plasmodium vivax*. Infection and Immunity *69*, 1084-1092.

Turner, L., Lavstsen, T., Berger, S.S., Wang, C.W., Petersen, J.E., Avril, M., Brazier, A.J., Freeth, J., Jespersen, J.S., Nielsen, M.A., *et al.* (2013). Severe malaria is associated with parasite binding to endothelial protein C receptor. Nature *498*, 502-505.

Vaidya, A.B., Morrisey, J.M., Zhang, Z., Das, S., Daly, T.M., Otto, T.D., Spillman, N.J., Wyvratt, M., Siegl, P., Marfurt, J., *et al.* (2014). Pyrazoleamide compounds are potent antimalarials that target Na+ homeostasis in intraerythrocytic *Plasmodium falciparum*. Nat Commun *5*, 5521.

van Eijk, A.M., Ayisi, J.G., Ter Kuile, F.O., Slutsker, L., Shi, Y.P., Udhayakumar, V., Otieno, J.A., Kager, P.A., Lal, R.B., Steketee, R.W., *et al.* (2007). HIV, malaria, and infant anemia as risk factors for postneonatal infant mortality among HIV-seropositive women in Kisumu, Kenya. J Infect Dis *196*, 30-37.

van Schaijk, B.C., Ploemen, I.H., Annoura, T., Vos, M.W., Foquet, L., van Gemert, G.J., Chevalley-Maurel, S., van de Vegte-Bolmer, M., Sajid, M., Franetich, J.F., *et al.* (2014). A genetically attenuated malaria vaccine candidate based on *P. falciparum* b9/slarp gene-deficient sporozoites. eLife *3*.

Vennerstrom, J.L., Arbe-Barnes, S., Brun, R., Charman, S.A., Chiu, F.C., Chollet, J., Dong, Y., Dorn, A., Hunziker, D., Matile, H., *et al.* (2004). Identification of an antimalarial synthetic trioxolane drug development candidate. Nature *430*, 900-904.

Volz, J., Yap, A., Sisquella, X., Thompson, J.K., Lim, N.T.Y., Whitehead, L.W., Chen, L., Lampe, M., Tham, W.-T., Wilson, D., *et al.* (2016). The essential invasion complex PfRh5/PfRipr/CyRPA is linked to injection of calcium into human erythrocytes by merozoites during invasion. Cell Host and MIcrobe *In Press*.

Wassmer, S.C., Taylor, T.E., Rathod, P.K., Mishra, S.K., Mohanty, S., Arevalo-Herrera, M., Duraisingh, M.T., and Smith, J.D. (2015). Investigating the Pathogenesis of Severe Malaria: A Multidisciplinary and Cross-Geographical Approach. Am J Trop Med Hyg *93*, 42-56.

Weiss, G.E., Gilson, P.R., Taechalertpaisarn, T., Tham, W.H., de Jong, N.W., Harvey, K.L., Fowkes, F.J., Barlow, P.N., Rayner, J.C., Wright, G.J., *et al.* (2015). Revealing the Sequence and Resulting Cellular Morphology of Receptor-Ligand Interactions during *Plasmodium falciparum* Invasion of Erythrocytes. PLoS Pathog *11*, e1004670.

White, N.J., Pukrittayakamee, S., Hien, T.T., Faiz, M.A., Mokuolu, O.A., and Dondorp, A.M. (2014a). Malaria. Lancet *383*, 723-735.

White, N.J., Pukrittayakamee, S., Phyo, A.P., Rueangweerayut, R., Nosten, F., Jittamala, P., Jeeyapant, A., Jain, J.P., Lefevre, G., Li, R., *et al.* (2014b). Spiroindolone KAE609 for *falciparum* and *vivax* malaria. N Engl J Med *371*, 403-410.

WHO (2015). The World Malaria Report http://wwwwhoint/malaria/publications/world-malaria-report-2015/report/en/ *ISBN 978 92 4 156515 8*

Wickham, M., Culvenor, J., and Cowman, A. (2003). Selective inhibition of a two-step egress of malaria parasites from the host erythrocyte. Journal of Biological Chemistry *278*, 37658-37663.

Wu, Y., Sinden, R.E., Churcher, T.S., Tsuboi, T., and Yusibov, V. (2015). Development of malaria transmission-blocking vaccines: from concept to product. Adv Parasitol *89*, 109-152.

Xin-zhuan, S., Heatwole, V.M., Wertheimer, S.P., Guinet, F., Herrfeldt, J.A., Peterson, D.S., Ravetch, J.A., and Wellems, T.E. (1995). The large diverse gene family *var* encodes proteins involved in cytoadherence and antigenic variation of *Plasmodium falciparum*-infected erythrocytes. Cell *82*, 89-100.

Yalaoui, S., Huby, T., Franetich, J.F., Gego, A., Rametti, A., Moreau, M., Collet, X., Siau, A., van Gemert, G.J., Sauerwein, R.W., *et al.* (2008). Scavenger receptor BI boosts hepatocyte permissiveness to *Plasmodium* infection. Cell host & microbe *4*, 283-292.

Yap, A., Azevedo, M.F., Gilson, P.R., Weiss, G.E., O'Neill, M.T., Wilson, D.W., Crabb, B.S., and Cowman, A.F. (2014). Conditional expression of apical membrane antigen 1 in *Plasmodium falciparum* shows it is required for erythrocyte invasion by merozoites. Cell Microbiol *16*, 642-656.

Yeo, T.W., Lampah, D.A., Kenangalem, E., Tjitra, E., Weinberg, J.B., Granger, D.L., Price, R.N., and Anstey, N.M. (2014). Decreased endothelial nitric oxide bioavailability, impaired microvascular function, and increased tissue oxygen consumption in children with falciparum malaria. J Infect Dis *210*, 1627-1632.

Zeeman, A.M., van Amsterdam, S.M., McNamara, C.W., Voorberg-van der Wel, A., Klooster, E.J., van den Berg, A., Remarque, E.J., Plouffe, D.M., van Gemert, G.J., Luty, A., *et al.* (2014). KAI407, a potent non-8-aminoquinoline compound that kills *Plasmodium cynomolgi* early dormant liver stage parasites *in vitro*. Antimicrob Agents Chemother *58*, 1586-1595.

Figure 1

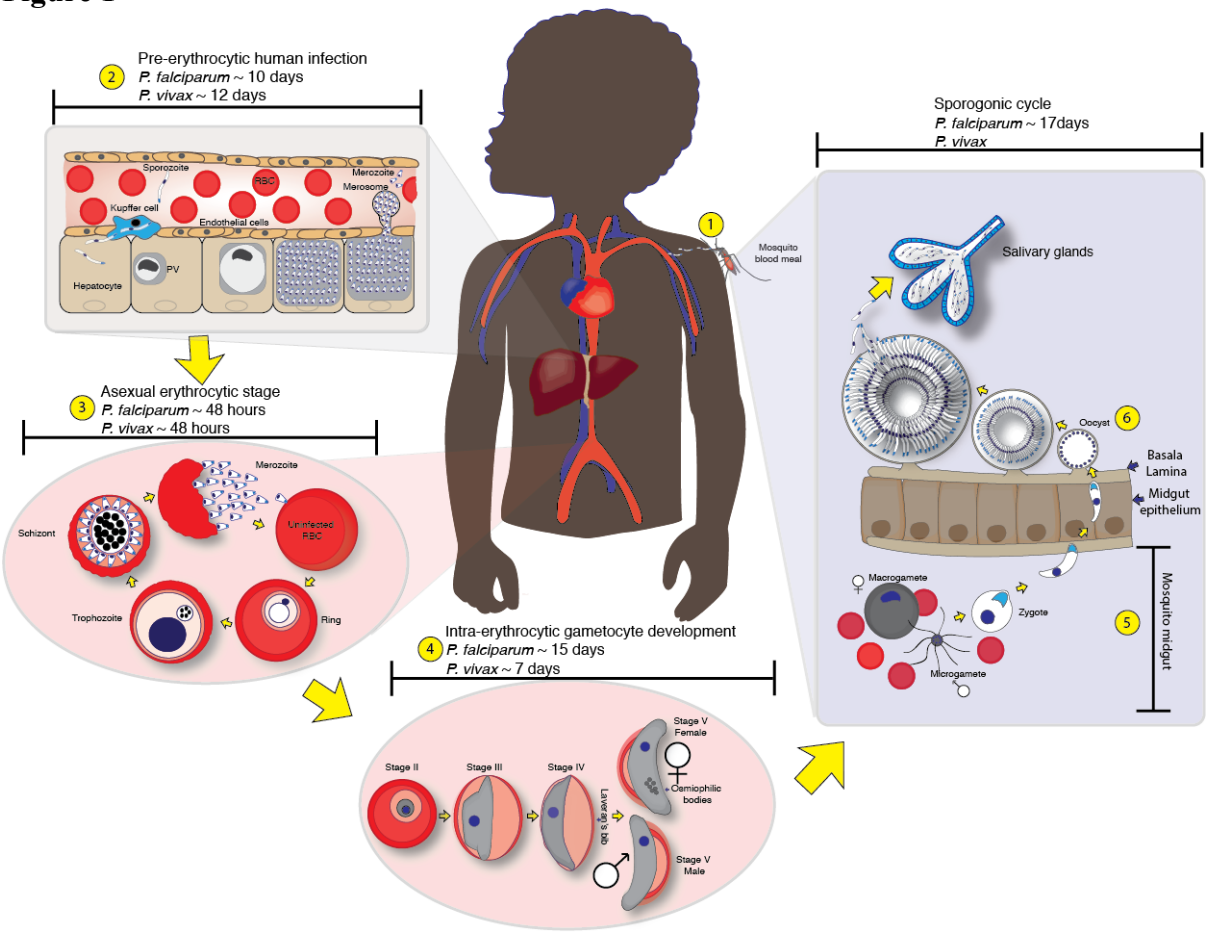

Pre-erythrocytic human infection
P. falciparum ~ 10 days
P. vivax ~ 12 days
Sporozoite
RBC
Merozoite
Merosome
Kupffer cell
Endothelial cells
Hepatocyte
PV
Asexual erythrocytic stage
P. falciparum ~ 48 hours
P. vivax ~ 48 hours
Merozoite
Schizont
Uninfected RBC
Trophozoite
Ring
Intra-erythrocytic gametocyte development
P. falciparum ~ 15 days
P. vivax ~ 7 days
Stage II
Stage III
Stage IV
Stage V Female
Gametocytes
Osmiophilic bodies
Stage V Male
Sporogonic cycle
P. falciparum ~ 17days
P. vivax
Mosquito blood meal
Salivary glands
Oocyst
Basala Lamina
Midgut epithelium
Macrogamete
Zygote
Microgamete
Mosquito midgut

Figure 2

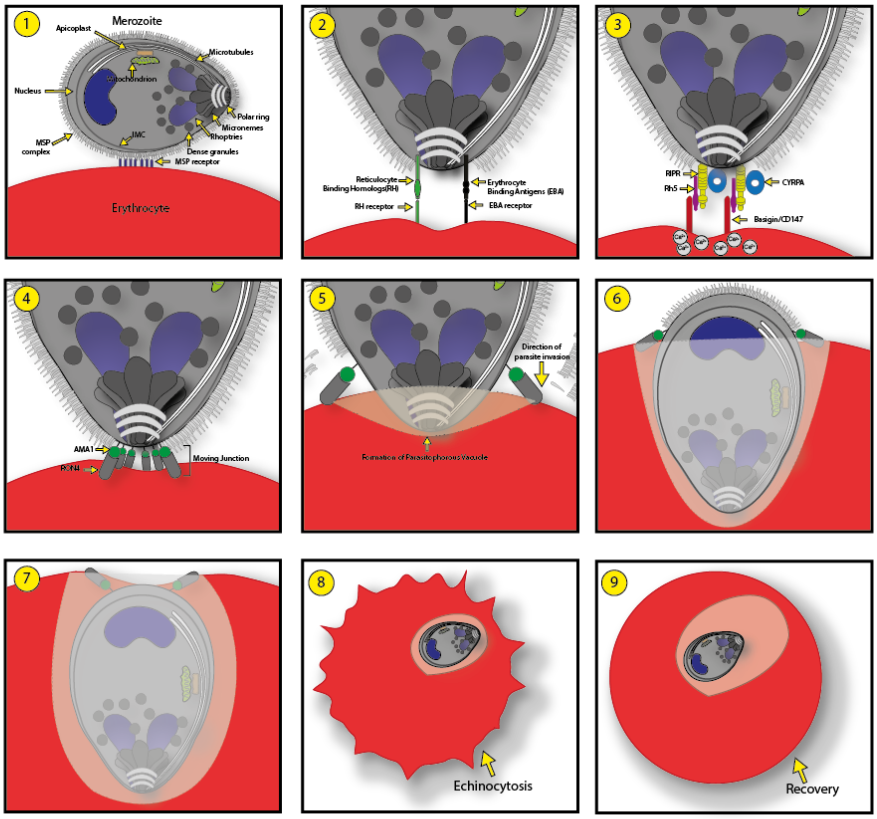

1
Merozoite
Apicoplast
Microtubules
Mitochondrion
Nucleus
Polar ring
Micronemes
Rhoptries
MSP complex
IMC
Dense granules
MSP receptor
Erythrocyte
2
Reticulocyte Binding Homologs(RH)
Erythrocyte Binding Antigens (EBA)
RH receptor
EBA receptor
3
RIPR
CYRPA
Rh5
Basigin/CD147
4
AMA1
Moving Junction
RON4
5
Direction of parasite invasion
Formation of Parasitophorous vacuole
6
7
8
Echinocytosis
9
Recovery

Figure 3

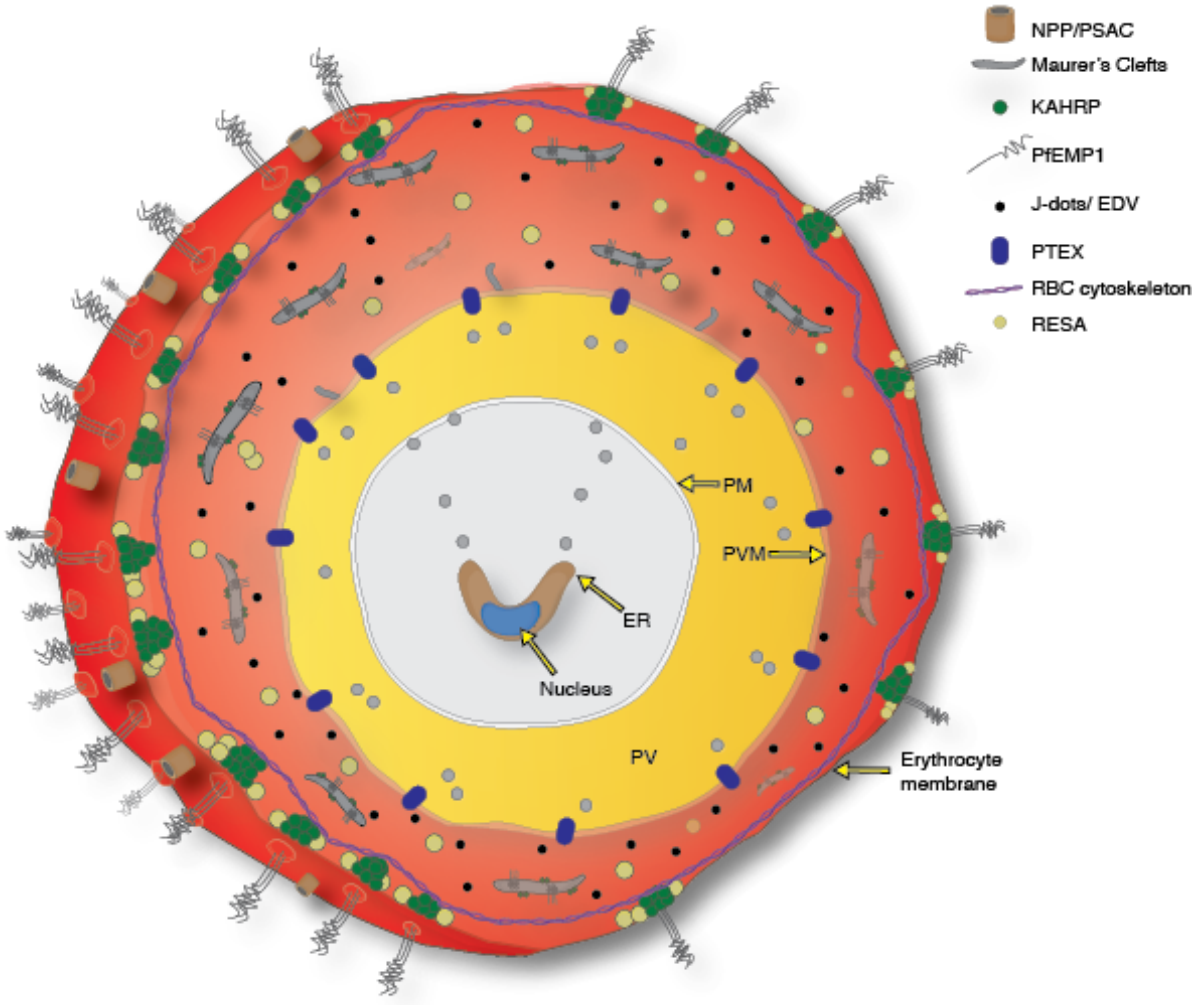

NPP/PSAC
Maurer's Clefts
KAHRP
PfEMP1
J-dots/ EDV
PTEX
RBC cytoskeleton
RESA
PM
PVM
ER
Nucleus
PV
Erythrocyte membrane

Figure 4

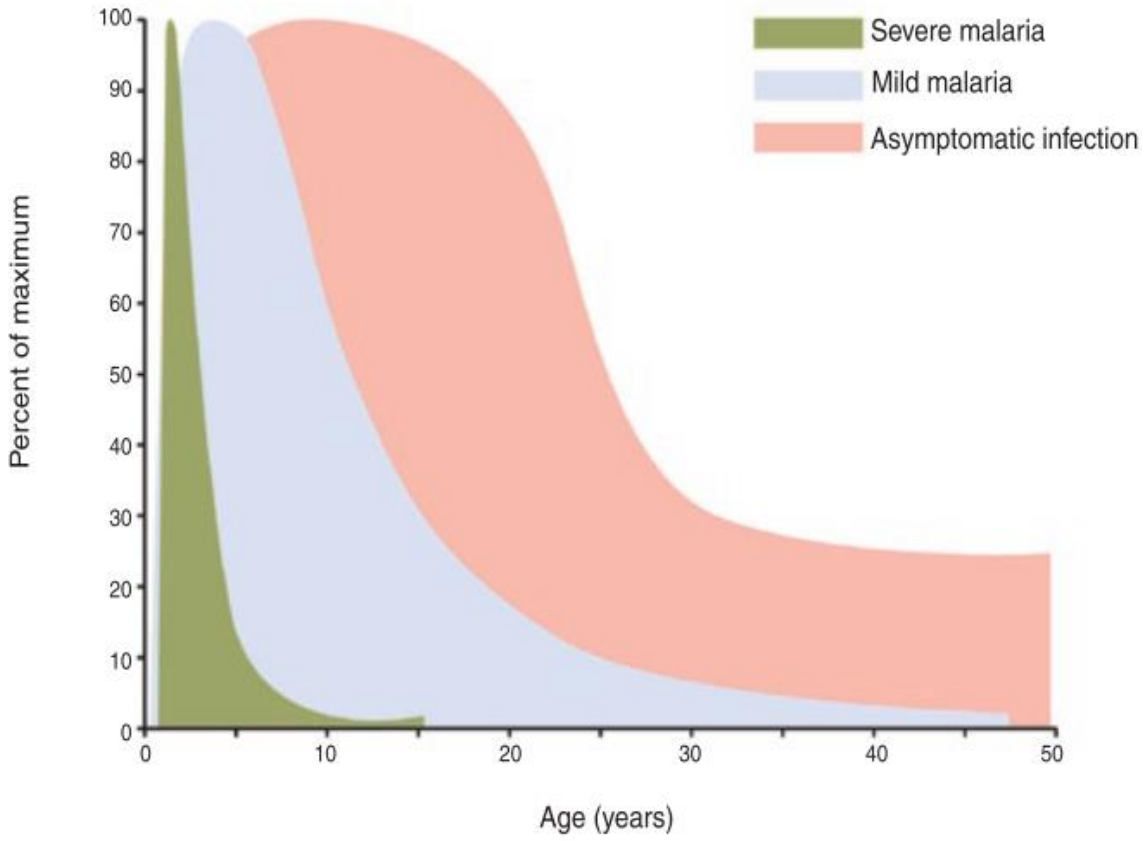
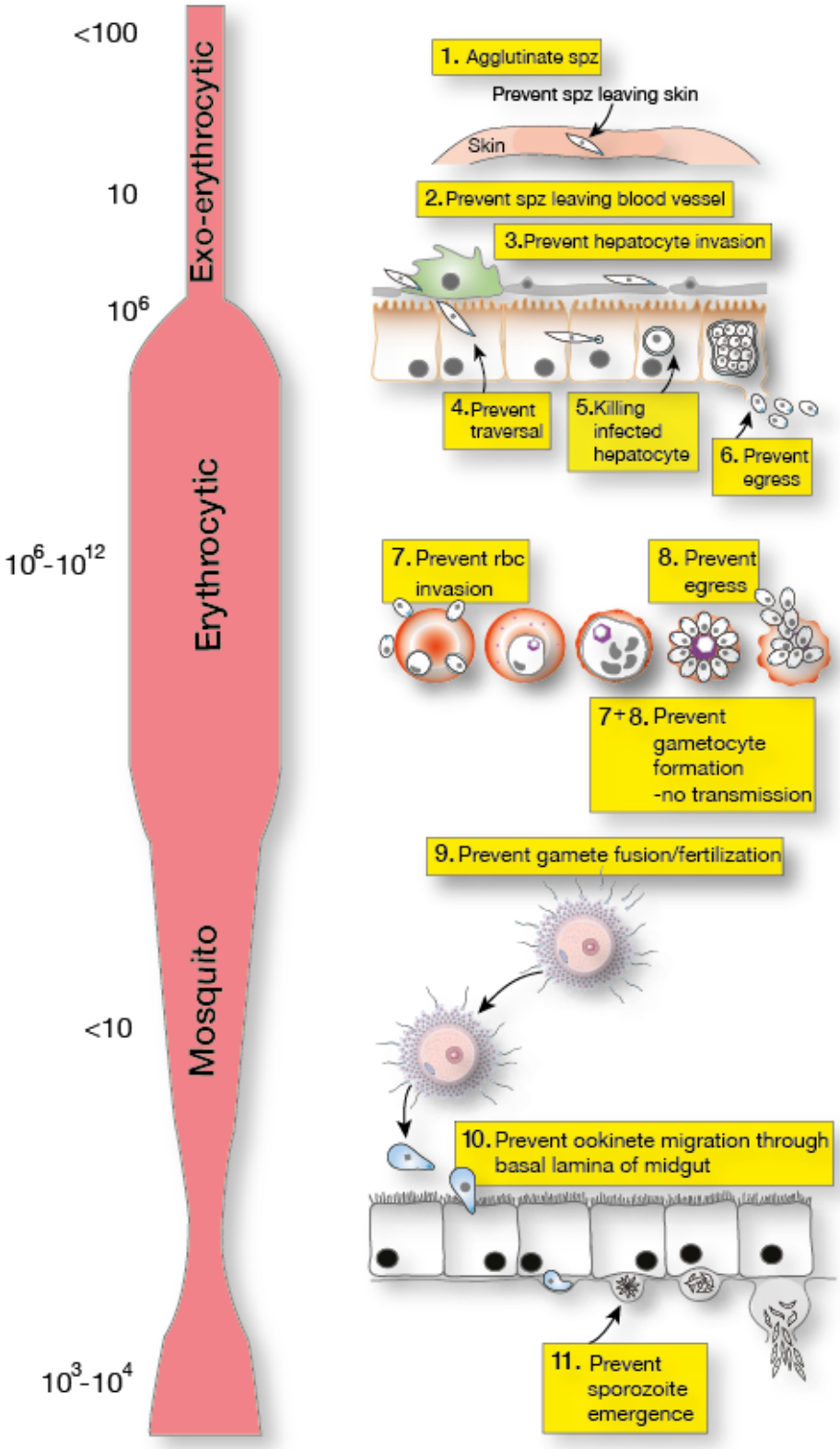

Severe malaria
Mild malaria
Asymptomatic infection
Percent of maximum
100
90
80
70
60
50
40
30
20
10
0
0
10
20
30
40
50
Age (years)

Figure 5


<100
10
10^6
10^6-10^12
<10
10^3-10^4
Exo-erythrocytic
Erythrocytic
Mosquito
1. Agglutinate spz
Prevent spz leaving skin
Skin
2. Prevent spz leaving blood vessel
3. Prevent hepatocyte invasion
4. Prevent traversal
5. Killing infected hepatocyte
6. Prevent egress
7. Prevent rbc invasion
8. Prevent egress
7+8. Prevent gametocyte formation -no transmission
9. Prevent gamete fusion/fertilization
10. Prevent ookinete migration through basal lamina of midgut
11. Prevent sporozoite emergence