There have been significant decreases in malaria mortality and morbidity in the last 10–15 years, and WHO estimated a 47% reduction in mortality between 2000 and 2013 [1]. Many malaria control measures, such as insecticide-treated bed nets (ITNs), indoor residual spraying (IRS), and treatment with artemisinin-based combination therapy (ACT), have contributed to this great achievement. In Africa, where the most virulent human malaria parasites, *Plasmodium falciparum*, still killed ~530,000 people (mainly children under 5 years old) in 2013, it is estimated that scale-up usage of ITNs made the biggest contribution to the reduction (68%), followed by ACT (19%) and IRS (13%) [2]. However, the emergence of mosquitoes and parasites resistant to existing control strategies has increased apprehension about future directions [2,3].

In July 2015, the most advanced malaria vaccine, RTS,S made by GlaxoSmithKline (GSK), received a positive opinion from European regulators for the first time [4]. The RTS,S vaccine is a pre-erythrocytic stage vaccine which is designed to prevent malaria infection and contains part of the circumsporozoite protein (CSP). The 3-year phase III efficacy study, which involved 8922 children (5–17 months old at enrollment) and 6537 infants (6–12 weeks), has shown 36.3% (95% confidence interval (CI): 31.8–40.5%) vaccine efficacy in children against clinical malaria and 25.9% (95% CI: 19.9–31.5%) in infants [5]. This major milestone in malaria vaccine development history has proved that an efficacious malaria vaccine is achievable. However, a more efficacious second-generation vaccine is needed and the duration of efficacy of the current RTS,S vaccine is concerning [6].

The feasibility of blood-stage vaccines has been supported by many epidemiological studies; people living in malaria endemic areas can acquire immunity against severe malaria initially, then clinical malaria [7]. Two passive IgG transfer studies in humans directly established that the immunity is at least in part mediated by antibodies. In the first passive transfer study conducted in The Gambia, children with acute malaria received purified IgGs from Gambian malaria-immune adults [8]. The parasite density dropped significantly from 10,000–230,000 parasites/μl to zero in 8 out of 12 children (the maximum of 80/μl in one child) by day 9, while transfusion of non-IgG fraction of the sera or IgG from malaria naive UK people had no effect. In the second study, IgG from African adults was inoculated to Thai patients with 4200–9000 parasites/μl [9]. The parasitemia went down to 8–90 parasites/μl between 33 and 113 h after the initial inoculation. The Thai study has shown that IgG from a different geographical location has the capacity to kill parasites in vivo. The mechanism of parasite killing by the antibodies has not yet been resolved, but if a blood-stage vaccine can elicit such effective antibodies in humans, the vaccine is likely to prevent clinical malaria. The Malaria Vaccine Technology Roadmap updated in November
2013 targets two strategic goals by 2030 [10]: (1) vaccines with >75% efficacy against clinical malaria and (2) vaccines that reduce transmission of the parasite and thereby substantially reduce malaria infection. A new blood-stage vaccine or a combination of vaccines against the blood-stage and pre-erythrocytic stages of malaria is needed to achieve the 75% goal. If the vaccine has a strong enough efficacy, it can also reduce transmission by significantly lessening the gametocyte numbers in humans.

The first part of this review summarizes the pros and cons of various assays and models which have been and will be used to predict efficacy of blood-stage vaccines. In the second part, blood-stage vaccine candidates which showed some efficacy in human clinical trials or controlled human malaria infection (CHMI) models are discussed. Then other candidates under clinical investigation are described in the third part, and novel candidates and strategies, which are not mentioned in the first three, are reviewed in the last part. This manuscript does not cover vaccines against pregnancy malaria or Plasmodium vivax vaccines since they are discussed elsewhere [11,12].

How to evaluate vaccine candidates

It is well acknowledged that developing a successful vaccine takes a long time and a great deal of money. In case of RTS,S, GSK initiated the development of this vaccine in the late 1980s, and GSK and the Bill and Melinda Gates Foundation have invested approximately $610 million to date [13]. Therefore, it is very important to establish a surrogate assay(s) and/or model(s), by which we can down-select or terminate an unsuccessful vaccine as soon as possible. By doing that we can focus on more promising novel vaccines. However, since none of the blood-stage vaccines have shown a strong efficacy in the field (i.e. either in phase II or III clinical trials), no assay/model can be established as a surrogate. Many assays and models have been utilized during the RTS,S preclinical and clinical studies, but recent data indicate that anti-circumsporozoite antibody titers are the best surrogate of protection based on the phase III study results [6]. At this moment, only a phase II trial is the best ‘surrogate’ assay for testing the efficacy of blood-stage vaccines, but we cannot reach a phase II trial without evaluating the vaccine candidates by some assays/models. Therefore, the following sections discuss pros and cons of each assay which has been (or will be) used for blood-stage vaccine development.

Enzyme-linked immunosorbent assay (ELISA), western blot, and immunofluorescence assay (IFA)

As described before, two human passive transfer studies clearly showed that antibodies are the principal contributors to anti-blood-stage parasite immunity in the field (either directly, in combination with other cells, or both). Therefore, many longitudinal (prospective) immuno-epidemiology studies have been conducted to find novel vaccine candidates or to add rational support for further development of existing candidates. Total IgG responses, IgG subclasses, and avidity of antibodies (e.g. using ammonium thiocyanate) were also assessed in many studies. ELISA is easy to perform in many laboratories and relatively easy to standardize compared to other biological assays which are described later. Previously only one or a few proteins were examined in a study, but protein microarrays (which can test more than 1000 proteins simultaneously) began to be applied to longitudinal studies [14]. In preclinical and clinical trials, ELISA is almost always performed to determine the immunogenicity of the test vaccines. However, there are several issues that need to be considered. First of all, the ELISA results depend on the quality of the recombinant proteins (or extracted proteins from parasites) used for ELISA. Indeed, there were two phase I trials conducted with PfCP2.9, which is a recombinant fusion protein of merozoite surface protein 1 (MSP1) and apical membrane antigen 1 (AMA1). The vaccine did induce antibody responses in vaccinees measured by ELISA with the vaccine protein, but the antibodies did not recognize parasites by IFA in one study [15] and did not show any activity in a biological assay, the growth inhibition assay (GIA) [15,16]; in contrast, many human trials have shown MSP1- and AMA1-based vaccines can induce functional antibodies as judged by GIA. In this sense, IFA or western blot using native proteins are better than ELISA with recombinant proteins, but it is not assured that IFA/western positive antibodies can recognize antigen expressed in live parasites, and IFA and western blot assays are not as quantitative as ELISA.

The correlations between immune responses measured by ELISA and clinical protection measured in longitudinal studies vary significantly depending on the study sites [17]. The differences could be caused by many factors: protein used for ELISA, ELISA methodology, endemicity, and parasite strains in the particular field site. Similarly the correlations between GIA results and clinical protection are controversial [18]. Therefore, unless an assay has been performed by multiple investigators in multiple field sites, it is questionable whether we can generalize the findings from one longitudinal
study. Another point that must be considered to interpret the data from cohort studies is correlation and causality. When an IgG response (or combination of responses measured by any assay) significantly associates with a reduction of clinical malaria risk in a longitudinal study, the data cannot prove causality, only correlation. For example, several studies have shown that longitudinal studies are extremely valuable to search for a novel potential candidate and a novel functional assay, which could eventually be a surrogate of vaccine-induced clinical protection. However, because of those limitations, I only discuss results from epidemiological studies in the following sections when it is critical.

**GIA/IIA**

The GIA or invasion inhibition assay (IIA) is one of the most widely used functional assays in blood-stage vaccine development. In general, parasites are co-cultured with either control or test antibodies, and % inhibition in parasite numbers (parasitemia) after the incubation is calculated. When parasitemia is measured just after merozoite invasion (usually within 20 h of invasion), the assay is called IIA, while when parasitemia is evaluated at a later time point (40–72 h after starting the culture), it is designated as GIA. If the mechanism of action of test antibody is only to prevent invasion of merozoites into uninfected erythrocytes, IIA and GIA should give the same % inhibition results. On the other hand, GIA can also measure the inhibitory effect on intraerythrocytic parasite development, and such a phenomenon was reported in the case of anti-MSP1 antibody [21]. While inhibitions in invasion, in growth, or both represent differences in parasite biology, in vaccine development as an actual ‘IIA’ is often called ‘GIA’ in many publications. In addition, antibodies which are known to block only parasite invasion are tested by GIA, instead of IIA. Therefore, for simplicity, I will use the terminology of ‘GIA’ in the following manuscript. There are many minor variations in GIA. For example, researchers usually use infected erythrocytes with late trophozoite or schizont stage parasites to initiate the assay, but purified merozoites are also used in some studies; determination of the final parasitemia may be done microscopically, by a flow cytometer, or by a parasite-specific enzymatic activity; parasites may be incubated with antibody less than one cycle (20–40 h) or two cycles (~72 h). A study reported that the final results could differ slightly depending on the methods and types of test antibodies [22].

GIA has been routinely performed in many different laboratories in the world, and it is easy to use different strains of parasites to evaluate the impact of polymorphisms in the target antigens. Therefore, these assays have been utilized not only in many animal immunization studies but also in many phase Ia trials, such as AMA1 [23–25], MSP1 [26], and erythrocyte binding antigen (EBA)-175 [27]. The important point is that the vaccines developed by different investigators and tested in different platforms can induce GIA-positive antibodies when the human antibodies were tested at the same or lower concentrations than those seen in their blood.

Despite the wide usage of GIA, there are two key questions remaining for GIA in vaccine development; one is whether GIA is suitable for trials in malaria-exposed populations. The second, more serious question is whether GIA is a useful assay to predict efficacy in the field. In terms of the first question, it is reported that GIA results could change depending on the population immunized. One example was that AMA1-C1 (a mixture of AMA1-FVO and AMA1-3D7 recombinant proteins) adsorbed on Alhydrogel could induce GIA-positive antibodies in US adults [23], but not in Malian adults [28], while elevations of anti-AMA1 antibody titers measured by ELISA were observed in both populations. Another example was FMP2.1 (AMA1-3D7 protein) formulated with AS02A adjuvant. Similar to the AMA1-C1/Alhydrogel vaccine, the FMP2.1/AS02A vaccine increased anti-AMA1 titers regardless of vaccinees, but increases in GIA activity were only observed in malaria-naïve adults [24], but not in malaria-immune adults [29]. Another phase Ib study revealed that the AMA1-C1/Alhydrogel vaccine could increase % inhibition in GIA in Malian children, but only in those who had no GIA activity before immunization (like a malaria-naïve population) [30]. In addition, children with higher anti-AMA1 titers at baseline have more ‘interfering’ antibodies, which could block GIA activity of affinity-purified human anti-AMA1 antibodies [31], and such ‘interfering’ antibodies were also observed in Malian adults [32]. The ‘interfering’ antibodies were malaria-specific IgGs, but the target antigen(s) has not been identified. Further investigation is required to determine whether the ‘interfering’ antibodies actually diminish the vaccine efficacy in the field, or are just an artificial observation with in vitro GIA. In either case,
interpretation of GIA results from vaccine trials in malaria-experienced individuals is complicated.

The second question for GIA is more important. As discussed above, ultimate proof or disproof cannot be done until a blood-stage vaccine shows a measurable efficacy in a phase II (or III) trial. However, many vaccine formulations, which can induce measurable GIA-active antibodies in humans, have not shown significant efficacy in either phase II trials or CHMI models. In one CHMI study, there was a significant inverse correlation between parasite multiplication rate (PMR; fold-increase of parasitemia per 48-h cycle) and GIA activity in AMA1 vaccinees \( (p = 0.02, n = 6) \) [33]. However, the significant correlation disappeared when two control volunteers were included in the analysis \( (p = 0.15, n = 8) \). One possible explanation is that GIA is not a surrogate assay, and the other is that the levels of GIA activity reached in human vaccinees were too low to show any efficacy. The latter possibility is partially supported by monkey challenge studies. When Aotus monkeys were immunized with AMA1-based vaccines and then challenged with P. falciparum parasites, all monkeys who were protected against the challenge showed \( >70\% \) inhibition in GIA before parasite challenge [34]. Another Aotus monkey challenge study with a MSP1-based vaccine showed that all protected monkeys had \( >80\% \) inhibition in GIA [35]. Since the GIA conditions in those two studies were different, it is difficult to compare the \% inhibition values directly, but both studies suggested that higher GIA activities might be required to show protection at least in the monkey challenge models. If it is also true in humans, a much stronger vaccine formulation needs to be developed.

Recently, Boyle et al published that some (but not all) IgGs from Kenyan and Papua New Guinea sera showed higher invasion inhibition in the presence of complement [36]. On the other hand, when Malian adults IgGs \( (n = 19) \) were tested with or without complement, none of the IgGs showed different \% inhibition (unpublished data). The difference might be explained by the methods utilized; Boyle’s IIA was done with purified merozoites, and our GIA with infected erythrocytes. The same group previously published that purified merozoite IIA showed higher \% inhibitions compared to the regular infected erythrocyte IIA when the same anti-AMA1 mAbs were tested [37]. In any case, the effect of complement in GIA/IIA needs to be investigated further.

**ADCI**

Antibody-dependent cellular inhibition (ADCI) assay is an assay to determine parasite-killing effects of soluble factors (including TNF-\( \alpha \)) released from human monocytes which are activated by a test antibody [38]. The African adults’ IgG used in the second passive transfer study showed a positive response in ADCI, but not in GIA [39]. Therefore, ADCI is considered as one of the potential surrogate assays. Since test antibodies may directly block the parasite invasion or growth (which can be measured by GIA) and monocytes may release killing factors without antibody stimulation, ADCI results are usually expressed as a specific growth inhibition (SGI) index, which is intended to exclude the inhibitory effect of monocyte alone and antibody alone. MSP2 [40], MSP3 [41], and glutamate-rich protein (GLURP) [42] vaccines induced ADCI-active antibodies in humans, and affinity purified human anti-serine repeat antigen 5 (SERA-5) antibodies showed positive ADCI [43]. Interestingly, all of those antigens have not been reported to induce GIA-active antibodies.

While ADCI assay is potentially a valuable assay for vaccine development, this assay is not easy to perform and it has been very difficult for many laboratories to execute this assay. The barrier in implementing ADCI assay could be partially explained by the heterogeneity of the human monocytes used. Depending on the subset of monocytes characterized by a series of surface markers, such as CD16, CD14, and CCR2, ADCI activities vary significantly [44]. Even when monocytes were collected from the same individual from different days, the SGI changed from 12.2% to 56.5% [45]. While there is a report attempting increase in the throughput of assay [46], so far ADCI with a monocyte cell line has not been successful despite efforts in many laboratories. To utilize this assay more widely, further definition and optimization are required.

**Phagocytosis/opsonization assay**

Opsonization and phagocytosis assays have not been widely utilized for antibody samples from clinical trials as yet, but several immuno-epidemiology studies have shown a correlation between phagocytosis activities and reduction in clinical malaria [47,48]. The phagocytosis assays loosely fall into four categories based on the parasites and phagocytic cells: whether the assay is performed with purified merozoites [47,48] or with infected erythrocytes [49,50] and whether THP-1 human monocyte cell line [47,49,50] or primary peripheral blood mononuclear cells [48,51] are used. The expression pattern of the target antigen determines the parasite source (either merozoites or infected erythrocytes) for a phagocytosis assay. The assay with THP-1 cells is considered to provide more reproducible results. However, the assay cannot cover the diversity...
of mononuclear cells in individuals and THP-1 cells do not express FcγRIII receptors [52].

Since it takes only 30–120 s for merozoites from egress to invasion [53] and merozoites lose their infectivity very rapidly at 37°C [54], whether phagocytosis of infectious live merozoites mediated by vaccine-induced anti-merozoite antibodies has a significant impact in vivo is debatable. On the other hand, parasite-derived antigens expressed on the surface of infected erythrocytes are exposed to human effector mechanisms for a much longer time. However, such antigens which are exposed to the human immune system are known to be highly variable and are called variant surface antigens (VSAs). Therefore, developing a cross-reactive VSA-based vaccine is extremely challenging [55]. Even in recent animal immunization studies, VSA-based (more specifically P. falciparum erythrocyte membrane protein 1, PFEPM1-based) vaccines only showed cross-reactivity to similar types of PFEMP1, but not for other types of PFEMP1 [56–58]. Therefore, it has been suggested that a combination of PFEMP1 antigen(s) and non-PFEMP1 antigen(s) is likely to be required to develop an effective vaccine [59], except in the case of VAR2CSA-based vaccines against pregnancy malaria (which are not covered in this review).

**Other antibody-based assays**

Since EBA-175 region II is a binding region of the EBA-175 molecule to the erythrocyte, the blocking activity of human antibodies induced by a EBA-175 region II vaccine was tested using recombinant protein and erythrocytes, in addition to the regular GIA in a phase I trial [27]. When other EBAs and reticulocyte binding-like homologue (Rh) antigens reach to the clinical development stage, the erythrocyte binding assay might be used more frequently in human trials. However, whether the binding assay provides any additional information beyond that obtained from GIA, in terms of predicting vaccine efficacy, needs to be explored.

In case of ADCI assay, monocytes are utilized as the effector cells. On the other hand, the antibody-dependent respiratory burst assay utilizes polymorphonuclear neutrophils (PMN), and production of reactive oxygen species by the PMN is measured, rather than parasite killing [60]. The assay has not been utilized for any human trials, and similar to the ADCI, significant donor-to-donor variations of PMN sources are reported [61].

In case of anti-PFEMP1 antibodies, three more antibody-based assays have been utilized in animal immunization studies: an agglutination assay (i.e. whether a vaccine-induced antibody blocks agglutination of infected erythrocytes) [62], a rosette disruption (inhibition) assay [49,58], and a binding (or adhesion) inhibition assay [56,63–65]. Extensive studies have sought to identify the specific receptor(s) of each PFEMP1 antigen (domain) [59]; for example, a domain cassette 4-type of PFEMP1 mediates binding to intercellular adhesion molecule 1 (ICAM-1), and a VAR2CSA-type to chondroitin sulfate A (CSA). Therefore, in PFEMP1-based vaccine development, if a target molecule is known to mediate agglutination, rosetting, and/or binding either to a specific receptor (e.g. ICAM-1, CSA) or a specific cell type (e.g. endothelial cells), an inhibition assay using a vaccine-induced antibody can be applicable. However, since there is no common receptor or a common phenotype (agglutination or rosetting) for all PFEMP1 molecules, the assay should be tailored for each PFEMP1-based vaccine. Furthermore, a conserved epitope(s), which covers all diversity in PFEMP1 molecules, has not been identified. Those assays have been beneficial to understand the natural immunity in the field. However, considering the diversity of PFEMP1, it is arguable whether the assays are useful for development of a blood-stage vaccine that can actually reduce clinical malaria (or a specific type of clinical malaria, e.g. severe malaria) in the field. Since no PFEMP1-based vaccine (other than those based on VAR2CSA for placental malaria) has reached (or soon will reach) clinical development, it will take longer to evaluate the importance of those assays for vaccine development.

**T cell-based assays**

Vaccine-induced T cell responses have been measured in multiple phase I trials with many different target antigens. The T cell-based assays include a proliferation assay (measuring proliferation of T cells against ex vivo immunogen stimulation) and measurements of immunogen-induced cytokine/chemokine (IFN-γ, IL-2, TNF-α, etc.) production by various methods (e.g. ELISPOT, intracellular cytokine staining, and ELISA) [66–70]. The accumulated data clearly show that blood-stage vaccines can induce T cell responses in humans. However, there is no strong evidence in humans that such T cell immunities induced by the blood-stage vaccinations work as an independent effector mechanism of protection (i.e. T cells by themselves or cytokine/chemokine released from the T cells directly kill blood-stage malaria) rather than to support antibody production and maintenance. If there is no independent mechanism, it is natural to assume that an antibody-based assay has a higher likelihood to be a surrogate than a T cell-based assay. In one human immunization study, four volunteers were inoculated with a low dose of P.
falciparum–infected erythrocytes and drug cured three times, then challenged again with the same *P. falciparum*–infected erythrocytes [71]. Since the investigators did not find anti-malarial antibodies in the volunteers, the results from this immunization study suggested that T cell immunity worked independently. However, a later study revealed that the level of residual drug was unexpectedly high at the time of the last parasite challenge [72]. The results from the latter study made the interpretation of results from the former study difficult.

**Monkey challenge model**

The monkey *P. falciparum* challenge model has been a useful tool to evaluate many different vaccine formulations and also has been used to find novel candidates [34,73–77]. Many monkey studies were conducted with Freund’s adjuvant to induce maximum immune responses, but Freund’s adjuvant cannot be injected into humans because of its toxicity. To make this model more valuable, several studies were conducted with human-applicable adjuvants or vaccine formulations, and showed protective effects in some studies [34,75,77]. Monkey challenge studies can be done with non-GMP grade vaccines, and in contrast to the human challenge model (described next), investigators can follow the animals for a longer time until the monkeys develop high parasitemia or anemia. Therefore, the monkey challenge model might be a better model to evaluate the immunity against clinical disease, more than anti-infection immunity. For the negative side, because of the restriction in animal numbers which can be used for vaccine development and growing ethical concerns for using non-human primates, it has been becoming difficult to perform the monkey challenge studies in many countries. Another limitation is that since *Aotus* or *Saimiri* monkeys are not natural hosts of *P. falciparum* parasites, only a handful of strains (e.g. FVO, FCH/4, FUP-SP) which are adapted to the monkeys can be used for the challenge. Extensive discussions of the monkey challenge model have been published elsewhere [78,79].

**Controlled human malaria infection**

The sporozoite challenge model has been broadly used for pre-erythrocytic vaccines. Since a pre-erythrocytic vaccine is designed to kill parasites before merozoite-stage parasites enter the blood stream, the sporozoite challenge model is an excellent model to evaluate efficacy. The same model has been applied to the combination of blood-stage and pre-erythrocytic stage vaccines [80–84], and also for pure blood-stage vaccines [68,85]. Other blood-stage vaccine trials involved blood-stage parasite challenges, instead of sporozoite challenges [33,86]. Several reviews have already described the difference between sporozoite challenge and blood-stage challenge [87–89], and the blood-stage challenge is considered to be a more suitable model for blood-stage vaccines. In both challenge models, participants need to be treated when the level of parasitemia becomes microscopically detectable (or earlier if a volunteer shows any symptoms). Therefore, in addition to the time to detectable parasitemia by smear, PMR (or parasite growth rate) are calculated in many trials to evaluate the vaccine effect more comprehensively [33,68,83,86]. There was a significant difference in median PMR between Gambian (2.4-fold/48 h) and UK (8.0-fold/48 h) adults, which indicates that if a vaccine can induce immunity such as seen in African adults, it may show significant reduction in PMR. One obvious limitation of this model is that the test vaccine needs to be safe and clear all regulatory and ethical standards before performing the challenge study, that is, we cannot use the model in preclinical trials. Another drawback was that only NF54 or 3D7 strains of parasites have been sufficiently standardized for inoculation into humans. To overcome this limitation, several groups have been working to expand the diversity of parasite challenges [90,91]. To date the human challenge model is considered to be the closest to a phase II trial, but several questions need to be resolved in the future: (1) whether we should make a Go or No-Go decision for a phase II trial based on the CHMI results and (2) how much reduction in PMR is required to show efficacy in the field.

**Humanized mouse model**

Since only limited laboratories can perform monkey or human challenge studies, and both of them are very expensive, testing the efficacy of blood-stage vaccines in a humanized mouse is one of the attractive alternatives if applicable. While significant progress has been made in the last 5–10 years [92], still a majority of the humanized mouse models require daily injection of human erythrocytes [93]. A study with a new humanized model where the mice were infused with human hematopoietic stem cells, instead of mature human erythrocytes, has been published [94]. While the mice could maintain human erythrocytes in their peripheral blood up to 4 months without daily injection, the level of human erythrocytes was <1% of total RBC (i.e. >99% of RBC were mouse erythrocytes) and the parasitemia in the total blood was only 3–5 parasites/μl. Since the humanized mice are basically immunodeficient mice, the mouse model could be used only for passive
transfer experiments until recently. Huang et al. have reported very recently that they successfully reconstituted human CD4+ T and B cell responses in transgenic mice, in which a Pf-CSP vaccine could elicit protective immunity against a challenge with chimeric sporozoites (rodent parasites expressing Pf-CSP) [95]. However, further improvements are required to completely reproduce immune responses to a vaccine in humans. As more improvements in the humanized mouse model occur, this model will be used more broadly for future blood-stage vaccine development.

**Vaccine candidates that showed efficacy in humans**

In this section, blood-stage vaccines that were reported to show some efficacy in human clinical trials or CHMI models are discussed, and the summary of those trials are presented in Table 1. All of the antigens described in this section are merozoite surface antigens or antigens secreted from merozoite, and they are considered to have critical roles during merozoite attachment and/or invasion of erythrocytes.

**Combination B**

Combination B vaccine contained MSP1 (K1 allele), MSP2 (3D7 allele), and RESA (FCQ-27/PNG allele) antigens and was formulated with Montanide ISA720 adjuvant in a phase II trial with 120 children (5–9 years old) [96]. The 120 individuals were first divided into two groups; children were given either sulfadoxine-pyrimethamine (SP) or a placebo 1 week before immunization. In each group (SP or non-SP), half of the children received the Combination B vaccine and the other half received placebo vaccine (adjuvant alone); then parasite density and clinical malaria were monitored. Among the SP group, there was no effect on parasite density. In contrast within the non-SP group, the vaccine significantly reduced parasite density (p = 0.024). While there was a ‘mathematically’ significant reduction in parasitemia in the SP group, the vaccine did not show a ‘biologically’ significant effect in both groups, viz. no efficacy against clinical malaria. When both SP and non-SP groups were combined, children who received Combination B vaccine had less chance to be infected with parasites with the 3D7 form of MSP2: 78 out of 359 (22%) PCR samples collected from placebo group, and 30 out of 360 (8%) from vaccine groups (p = 0.04). The number of participants in each group was small (n = 30 each). This is the first blood-stage vaccine showing an allele-specific effect in malaria-exposed children. While the safety and immunogenicity results of a phase I study with MSP2-C1 (combination of 3D7 and FC27 allelic forms of MSP2) was reported in 2011 [40], according to the WHO malaria rainbow table [100] and ClinicalTrials.gov [101], no human trials with Combination B vaccine or other MSP2-based or RESA-based vaccines are planned in the near future.

**Table 1. Blood-stage vaccine candidates that showed significant effects in humans.**

| Trial | Vaccine formulation | Main outcome | Ref |
|-------|---------------------|--------------|-----|
| **Combination B (MSP1, MSP2, and RESA)** | | | |
| Phase Ib | *E. coli* expressed recombinant MSP1 (K1 strain), MSP2 (3D7), and RESA (FCQ-27/PNG) proteins with Montanide ISA720 adjuvant | No clinical protection, but strain-specific reduction in malaria infection: 78 out of 359 (22%) PCR samples showed 3D7 dimorphic form of MSP2 in the control groups, while 30/360 (8%) in the vaccine groups | [96] |
| **AMA1** | | | |
| Phase Ib | *E. coli* expressed recombinant AMA1 (3D7) protein with GSK adjuvant | No clinical protection, but strain-specific reduction in malaria cases. In 22 episodes (out of 271 total episodes observed during the trial) infected with AMA1-3D7 type parasites, 16 cases occurred in the control group, and another 6 cases in the vaccine group. | [97] |
| Phase Ila | *E. coli* expressed recombinant AMA1 (3D7) protein with GSK adjuvant | No significant difference in prepatent period or parasite growth rate after sporozoite challenge. However, significantly lower cumulative parasitemia during Day 7–9 after challenge in the vaccine group (n = 10) as compared to the unvaccinated infectivity control (n = 6). | [68]b |
| **MSP3** | | | |
| Phase Ib | MSP3 long synthetic peptide with aluminum hydroxide adjuvant | Significant reduction in risk of clinical malaria: 1.2 (15 µg dose) and 1.9 (30 µg dose) cases per 100 days per person in the vaccine groups (n = 15 each) while 5.3 in the control group (n = 15) | [98] |
| **SE36 (SERAS-5)** | | | |
| Phase Ib | *E. coli* expressed recombinant SERAS-5 (Honduras-1) protein with aluminum hydroxide adjuvant | Significant reduction in risk of clinical malaria: Hazard ratio = 0.26 after adjustment of age and gender: the vaccine group (n = 66) was compared to the control group (n = 16) and newly enrolled unvaccinated individuals (n = 50) | [99] |
| **MSP1** | | | |
| Phase Ila | Recombinant chimpanzee adenovirus 63 (ChAd63) and modified vaccinia virus Ankara (MVA) vectors encoding MSP1 | Significant difference in prepatent period after sporozoite challenge (n = 3 in the vaccine group and n = 6 in control) in the initial study, but not in the second study (n = 9 in vaccine and n = 6 in control) | [85] |

*Clinical trials which were conducted with a multistage vaccine(s) are not included.

bThere were other vaccine groups in the trial, but only one group which showed a significant effect is shown.
AMA1

While many phase I trials have been conducted with AMA1 vaccines, only two phase II trials have been completed so far. One of the studies involved AMA1-3D7 protein adjuvanted with AS02a (FMP2.1/AS02a) and was tested in 1–6 year Malian children [97]. A total of 383 children were followed completely for 240 days. Similar to the phase II trial with Combination B vaccine, there was no significant impact on clinical malaria. However, when only children who were infected with AMA1-3D7 type parasites (determined by cluster 1 loop of domain I sequences, the most polymorphic region in AMA1 molecule) were analyzed (22 episodes out of 271 episodes observed in the trial), there was a significant effect by the AMA1 vaccine: out of the 22 clinical episodes, 16 occurred in the control group and 6 in the vaccine group \( p = 0.03 \). The follow-up study showed no strain-specific protection in the next year [102]. Another phase II trial with a mixture of AMA1-3D7 and AMA1-FVO formulated on Alhydrogel in 2- to 3-year-old children (279 children were followed completely for 154 days) showed no impact on clinical malaria [103] or strain-specific protection [104].

In two human homologous sporozoite challenge trials, small, but significant, effects were observed in terms of cumulative parasitemia [68] or PMR [83]. However, neither study showed significant delay in treatment time (i.e. time to reach a level of parasitemia detected by light microscopy).

AMA1 is a highly polymorphic protein, and the phase II trial with FMP2.1/AS02a showed allele-specific protection. The GIA result from a phase I study indicated that a mixture of AMA1-3D7 and AMA1-FVO is unlikely to cover the variations in the field [105]. Therefore, many investigators have been trying to overcome the polymorphic issues for future AMA1-based vaccines. In animal immunization models, mixtures of 4 or 5 AMA1 proteins [106–108] could induce strain-transcending antibodies as judged by GIA. A rabbit study indicated the possibility that a modification of immunization strategy may further improve the cross-reactivity (i.e., immunize different AMA1 proteins sequentially, rather than inject the mixture of proteins every time) [109]. Another unique approach is to generate chimeric AMA1 proteins (DiCo) which could cover the majority of polymorphisms in the field by combining 3 DiCo proteins. The results of a non-human primate study were promising [110], and a phase I trial is underway (ClinicalTrials.gov Identifier NCT02014727). One more unique approach is to mutate major polymorphic residues to alanine, glycine, or serine [111]. While the chimeric AMA1 induced more cross-reactive antibodies judged by GIA, the levels of inhibition were lower compared to the antibodies raised with non-chimeric AMA1 for the same strains of parasites.

Researchers also have been attempting to generate more potent AMA1-based vaccines. AMA1 and rhoptry neck protein (RON) 2, 4, and 5 form a complex during merozoite invasion [112]. A recent animal immunization study suggests co-injection of AMA1 and RON2 may improve the efficacy of responses to homologous parasites [113]. Further study is required to determine whether the enhancement occurs in humans and whether the strategy works with AMA1-mixtures or DiCo vaccines.

MSP3 and GMZ2

MSP3-based vaccines could induce protective immunity in a monkey challenge model [114]. In addition, vaccine-induced human anti-MSP3 antibodies showed ADCI activity in vitro and killed P. falciparum parasites injected to humanized SCID mice in the presence of human monocytes [41,115]. While the efficacy was not tested in a phase II trial, efficacy was reported from a phase Ib trial in 1- to 2-year-old children conducted in Burkina Faso [98]. In the trial, groups of 15 children received either 15 μg of MSP3 long synthetic peptide (MSP3-LSP), 30 μg of MSP3-LSP, or control hepatitis B vaccine. The incident rates of clinical malaria in the MSP3 groups were significantly lower than that in control group (1.2 cases per 100 days for 15 μg MSP3, 1.9 for 30 μg MSP3, and 5.3 for control, \( p = 0.01 \)). Another vaccine, GMZ2, which contains MSP3 and GLURP is also under investigation [116–119]. The GLURP itself induced ADCI active antibodies in humans [42]. According to the WHO malaria rainbow table [100], both MSP3 and GMZ2 are the only two blood-stage candidates under phase IIb evaluation (no information in ClinicalTrials.gov [101]).

SE36

SE36 vaccine contains a part of SERA-5, results from one phase Ia trial [120] and from one phase Ib trial in Uganda [99] have been reported. In a part of phase Ib trial, 66 individuals (6–20 years old) received SE36 vaccine and were followed for clinical malaria episodes between 130 and 365 days post-second vaccination. In addition to the 16 individuals who were enrolled from the beginning and received saline (instead of SE36), the investigators newly enrolled 50 individuals before the clinical follow-up as the control group (\( n = 66 \) total) to increase the power
of the study. After adjustment for age and gender, the risk of parasitemia ≥ 5000 parasites/µl plus fever was significantly lower in the SE36 group compared to the control group (hazard ratio = 0.26 (95% CI, 0.10–0.61); p < 0.01). While there is no plan for a phase II trial (according to WHO malaria rainbow table [100] and ClinicalTrials.gov [101]), the efficacy of SE36 ought to be confirmed by a phase II trial.

MSP1

Multiple monkey challenge models showed MSP1-based vaccines could induce protective immunity in monkeys [35,121,122], and MSP1 is one of the components of Combination B vaccine as discussed above [96]. Only one phase II trial with pure MSP1-based vaccine has been conducted until today. The phase II trial conducted with MSP1,3D7 vaccine adjuvanted using AS02 in Kenyan children showed no significant effect on clinical malaria [123]. The significant effect of a MSP1 vaccine was only observed in one phase Ila trial where adults were immunized with chimpanzee adenovirus 63 (ChAd63) followed by modified vaccinia virus Ankara (MVA) [85]. While the number of vaccines was very small (n = 3 in the vaccine group, and n = 6 in control), there was a significant delay to the time to diagnosis by microscopy (p = 0.035) after sporozoite challenge. However, when the same ChAd63/MVA vaccine was tested in another phase Ila trial (n = 9 for a vaccine group and n = 6 in control), the MSP1 vaccine showed no significant effect (p = 0.13) [85].

It is likely that a stronger adjuvant and a new immunization strategy are required to make an efficacious MSP1-based vaccine.

Vaccines against multistage parasite antigens

NYVAC-PF7 is an attenuated vaccinia virus containing genes encoding candidates from multiple stages: pre-erythrocytic (CSP, SSP, and LSA1), blood-stage (MSP1, AMA1, and SERA), and mosquito-stage (Pfs25) antigens. When the volunteers were challenged with sporozoites (n = 35 in two doses of vaccine groups and n = 8 in the control group), there was a significant delay in prepatent period [81]. PMR was not measure in the trial. A significant effect by another multistage vaccine was also reported. PEV3A vaccine included peptides from CSP (pre-erythrocytic) and AMA1 (blood-stage) [83]. While there was no significant difference in prepatent period, PMR in PEV3A vaccinated group (n = 5, 5.7 parasites per ml per cycle) was significantly lower than that in the control (n = 5, 8.7 parasites per ml per cycle). However, since vaccines which contained only pre-erythrocytic antigens or blood-stage antigens were not tested in both studies, it is practically impossible to estimate how much (or any) protective effects were elicited by the blood-stage antigens.

Other vaccine candidates under clinical development

EBA175

A cysteine-rich second region of EBA-175 (EBA-175-RII) vaccine has been tested in a phase Ia trial, and the vaccine induced GIA-active antibodies. In a recent rabbit study, antibodies against more conserved regions of EBA-175 (regions III–V) showed stronger and more strain-transcending activities judged by GIA [124], while the functional activity of regions III–V is unknown. Further investigation is required to reveal whether the region III–V vaccine is better than the RII vaccine in humans. Since different field parasites show different protein expression levels of EBA and Rh proteins (EBA-140, EBA-175, EBA-181, RH1, and RH2 were tested in the study) [125], all of which are involved in redundant merozoite invasion pathways, a combination with other antigen(s) is likely to be required to show efficacy in the field.

P27A

P27A is a part of Trophozoite exported protein 1 (Tex1, previously called hypothetical protein PFF0165C). The Tex1 antigen was found by a unique approach, that is, based on the α-helical coiled coil structure of the molecule [126]. In contrast to other blood-stage candidates, Tex1 is not a merozoite protein, and locates at Maurer’s clefts in infected erythrocytes [127]. Human affinity-purified P27A-specific antibody and rabbit anti-P27A antibody showed ADCI activities [126,128], and the first phase Ia and Ib trial with Alhydrogel or GLA-SE adjuvants was completed in July 2015 (ClinicalTrials.gov Identifier NCT01949909). Several other antigens tested in the structure-based screening study showed ADCI activities [126], and they are also interesting candidates for further investigation.

RH5

RH5 is one of the reticulocyte binding-like homologue (Rh) proteins, and an RH5 vaccine could induce
strain-transcending antibodies in animals judged by GIA [129]. In addition, while the antibody levels in malaria-exposed individuals are low compared to other merozoite antigens [129,130], affinity-purified human anti-RH5 IgGs also showed GIA activity [130,131]. The low immunogenicity in humans may explain the reason why there are very limited polymorphisms in the molecule [132]. In an Aotus monkey study where the monkeys were immunized with the 3D7 sequence of RH5 using human-compatible vaccine formulations (ChAd63 vaccination followed by either MVA boost, or recombinant RH5 protein-Abisco-100 adjuvant boost), the vaccine induced a protective effect against heterologous FVO parasite challenge [133]. A phase Ia trial with ChAd63-MVA vaccines is ongoing (ClinicalTrials.gov Identifier NCT02181088).

In addition to studies with RH5 as a stand-alone vaccine candidate, many animal immunization studies have been conducted using mixtures of vaccines including RH5. For example, *P. falciparum* RH5 interacting protein (PfRipr) [134] and cysteine-rich protective antigen [135], both of which make a complex with RH5 during the merozoite invasion, can induce GIA-active antibodies by themselves and in combination with RH5. Furthermore, other merozoite antigens, such as EBA-175, RH1, RH2, RH4, AARP (apical asparagine-rich protein) and Pf38, have also been evaluated with RH5 [131,134–137], and the vaccines induced cross-reactive functional antibodies judged by GIA. Of interest, some combinations of IgGs showed synergistic invasion inhibition in GIA [135–137]. At this moment, it is not clear whether such synergistic protective effects can be observed *in vivo*.

**Other vaccine candidates**

In addition to the candidate antigens described above, many more potential candidates have been proposed from longitudinal cohort studies [14,138,139]. *P. falciparum* schizont egress antigen-1 (PfSEA-1) is one of the novel candidates found from cohort studies [140]. Similar to the P27A (Tex1), PfSEA-1 is not a merozoite antigen and localizes at the parasitophorous vacuole membrane, Maurer’s clefts, and the inner leaflet of the erythrocyte membrane. The anti-PfSEA-1 antibody prevents parasite egress rather than merozoite invasion. Anti-Pf332 antibody is likely to work with a similar mechanism [141], while a recent study suggested it might also block parasite growth through a different mechanism [142]. The data from P27A, PfSEA, and Pf332 studies indicate that not only merozoite proteins or VSAs but also antigens expressed within infected erythrocytes could be targets of blood-stage vaccines. Many other candidates, which are not described in this review, are also known to induce GIA-active antibodies at least in animal immunization studies, such as MSP4 [143], EBA140 [144], RON3 [144], GAMA (glycosylphosphatidylinositol – anchored micronemal antigen) [145], EBL-1 (erythrocyte-binding ligand-1) [146], MSPDBL1 and 2 (merozoite surface protein Duffy binding-like protein) [147,148], and RALP1 (leucine zipper-like protein 1) [149].

Several clinical trials have already been conducted with a mixture of multiple antigens (multiple stages) in different platforms, for example, AMA1+MSP1 [85,150], MSP1+EBA175 [151], CSP+AMA1 [82,84,152], and CSP +MSP2 [80]. Results from other multistage vaccines in animal immunization studies are also promising [153–155]. More multistage or multi-antigen vaccines are likely to be explored in the future. However, caution should be taken in such vaccines because antigenic competition has been observed in non-human primates and humans with AMA1+MSP1 vaccines [74,85]; that is, the mixture vaccine induced lower titers compared to the single antigen vaccine tested in the same study.

While it has not reached to the clinical trial stage, a group of researchers are investigating the possibility of using chemically attenuated blood-stage parasites as a vaccine [156], as a promising protective effect was observed by intravenous inoculation of attenuated sporozoites in humans in the case of a pre-erythrocytic vaccine [157].

**Expert commentary**

There are several considerations to accelerate future blood-stage vaccine development. As mentioned above, none of the assays/models has been proven as a surrogate of protection and no blood-stage vaccines have shown strong efficacy in a large phase II (or III) trial. Therefore, novel antigen discovery should be continued with any approach available (e.g. identify immune-correlates with clinical malaria in a longitudinal cohort study, structure/sequence-based predictions). However, since resources are limited, the functional activity of antibody (and cellular immunity if applicable) against the novel antigen should be evaluated promptly using an assay/model if live human parasites (or transgenic parasites expressing the human antigen). If the novel antigen induces only a weaker activity than an existing candidate(s), the novel candidate may have a lesser chance to be a successful vaccine, unless it can induce a synergistic effect with other candidates. If no robust functional activity of the novel vaccine-induced antibody is detected by an *in vitro*
assay, the investigators should consider the risk that vaccine development with the target molecule will be extremely challenging; viz. one needs to down-select vaccine formulations/adjuvants etc. without a reliable decision-making tool in the preclinical development stage and phase I trials. With new candidates which can induce functional antibodies and/or protection in a challenge model, a molecule that is known to be polymorphic and/or functionally redundant should be graded lower than a molecule which is non (or less)-polymorphic and/or functionally nonredundant, except when there is a methodology to overcome the issue.

Based on the published human trial data, the level of antigen-specific antibody which can be elicited by a vaccine in humans is expected to be somewhere between ~50 and several hundred μg/ml at the peak (i.e. 2–4 weeks after the final immunization), regardless of population immunized (malaria naïve or immune, children or adults), antigen (e.g. AMA1, MSP1, RTS,S), adjuvant (e.g. AS01, AS02, Cpg), or vaccine platform (e.g. recombinant protein, ChAd63/ MVI) [26,68,70,102,105,123,150,158–160]. Without a major breakthrough in vaccinology (e.g. a completely new strategy, a new class of adjuvant), one of the crucial aspects in vaccine development is to use the several hundred μg/ml of antibody efficiently. Therefore, I feel a polymorphic and/or functionally redundant molecule has a lesser chance to be a successful vaccine unless the novel candidate can induce a strong parasite killing effect at very low concentrations of antibody. In line with this consideration, novel vaccines that only contain critical epitopes ought to be investigated further. Using functional monoclonal antibodies, chimeric antigens and other methodologies, researchers have tried to identify critical epitopes in several existing candidate molecules [43,106,108,161–163]. Since the highest limit of antigen-specific antibody concentration is likely to be set, that is, a few hundred μg/ml (unless there will be a major break-through), it is also important to determine vaccine-induced antibody level in a μg/ml-scale, rather than ‘antibody titer’ or ‘antibody units’, in a human trial. By doing that, the investigator could estimate whether there is any room to improve the immunogenicity (e.g. change immunization schedule and/or adjuvant to reach the few hundred μg/ml level) or consider switching to a new candidate/strategy.

One of the other important keys for the vaccine development is to increase capacity for performing phase Iib (and Iia) trials and test a promising candidate in humans as soon as possible. Many blood-stage candidates have been shown to induce functional antibodies in animals, and human affinity purified IgGs also have shown functional activities judged by GIA, ADCI assay, or other assays. In addition, some vaccines can induce protective immunity in monkey challenge models. However, significant efficacies of blood-stage vaccines have been observed only in small phase I or Ila clinical trials (or in small subsets of phase IIb trials). Therefore, we should prove or disprove the vaccine efficacy in a phase Ila or IIb trial as quickly as possible rather than spending a great deal of time and effort in animal and preclinical studies once a promising candidate (or a combination of candidates) is identified. The results from phase II trials provide strong feedback for further vaccine development, that is, for Go and No-Go decisions, which assay(s) should be used, and what level of (functional) activity needs to be reached.

Not only the peak immune response, the longevity of responses is likely to determine the vaccine efficacy in the field. However, at this moment, there is no universally accepted strategy which maintains ‘vaccine-induced immunity’ for a long time, and which ‘vaccine-induced immunity’ should be measured as the surrogate of ‘protection’. A further complication is that there is no consensus on the best indicator of ‘protection’ in a phase Ila and IIb trials with a blood-stage vaccine. In epidemiology studies, different (or multiple) measurements have been reported (e.g. time to first malaria episode, risk of clinical cases per time per person). The selection of the ‘clinical protection’ readout(s) needs to be determined based on a target product profile of a vaccine, but if we aim to make ‘vaccines with >75% efficacy against clinical malaria (The Malaria Vaccine Technology Roadmap [10]),’ the vaccine should show a significant effect in any measurements.

Five-year view

I expect results from AMA1-DiCo, GMZ2, SE36, MSP3, P27A, and RH5 clinical trials will be available in the near future, and the data will guide further blood-stage vaccine development. Several other novel candidates described above may reach phase I or Ila trials in the next 5 years. In addition, it is likely that more development efforts will be focused on multi-antigen and multistage vaccines. Not only such ‘broader’ approach but also a ‘deeper’ approach (i.e. epitope specific approach) for each target antigen is also anticipated. For the host side, whole transcriptional analysis to identify biomarkers of protection is being applied to a pre-erythrocytic vaccine [164]. Once a blood-stage vaccine shows clear efficacy in a phase Ila (or IIb) trial, transcriptional analysis in the hosts will be explored further. In addition, further studies with human and humanized monoclonal antibodies (e.g. a passive transfer study with these antibodies in
a CHMI model) will be conducted to explore the interaction with the parasites in the human host.

Acknowledgement

I wish to acknowledge Dr. Carole Long for her valuable comments on the manuscript.

Financial & competing interests disclosure

This work was supported by the Intramural Program of the National Institutes of Health, National Institute of Allergy and Infectious Diseases. The author has no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

Key issues

- No in vitro assays or challenge models have been shown to be a surrogate of protection in the field for any blood-stage vaccines.
- However, GLA/IA and ADCl assays are used as function assays in many preclinical and clinical studies.
- Monkey parasite challenge model has been a useful tool to evaluate blood-stage vaccines in preclinical stages.
- Parasite challenge model, especially blood-stage challenge, in phase IIa trials is valuable and is becoming more widely used.
- Only Combination B, AMA1, MSP3, and SE63 vaccines were reported to show detectable levels of efficacy against either total or allele-specific parasites in humans.
- However, the size of the clinical trials (or subsets of the population analyzed in the clinical trials) was less than 70 per arm. A larger trial is necessary to confirm these findings.
- Many potential novel candidates have been identified in the last 5–10 years, and several novel vaccines (P27A, RHS, etc.) are under clinical investigation.
- Multi-allele, multi-antigen, and/or multistage vaccines need to be investigated further.

References

Papers of special note have been highlighted as
- of interest
- of considerable interest

1. WHO. World Malaria Report 2014; [cited 23 Oct 2015]. Available from: http://www.who.int/malaria/publications/world_malaria_report_2014/en/

2. Bhatt S, Weiss DJ, Cameron E, et al. The effect of malaria control on Plasmodium falciparum in Africa between 2000 and 2015. Nature. 2015;526:207–211.

3. White NJ, Pukrittayakamee S, Hien TT, et al. Malaria. Lancet. 2014;383:723–735.

4. Kaslow DC, Biemaux S. RTS, S: toward a first landmark on the Malaria Vaccine Technology Roadmap. Vaccine. 2015 pii: S0264-410X(15)01337-7. doi:10.1016/j.vaccine.2015.09.061.

5. RTS,S Clinical Trials Partnership. Efficacy and safety of RTS,S/AS01 malaria vaccine with or without a booster dose in infants and children in Africa: final results of a phase 3, individually randomised, controlled trial. Lancet. 2015;386:31–45.

6. White MT, Verity R, Griffin JT, et al. Immunogenicity of the RTS,S/AS01 malaria vaccine and implications for duration of vaccine efficacy: secondary analysis of data from a phase 3 randomised controlled trial. Lancet Infect Dis. 2015 pii: S1473-3099(15)00239-X. doi:10.1016/S1473-3099(15)00239-X.

7. Marsh K, Kinyanjui S. Immune effector mechanisms in malaria. Parasite Immunol. 2006;28:51–60.

8. Cohen S, McGl, Carrington S. Gamma-globulin and acquired immunity to human malaria. Nature. 1961;192:733–737.

9. Sabchareon A, Burnouf T, Ouattara D, et al. Parasitologic and clinical human response to immunoglobulin administration in falciparum malaria. Am J Trop Med Hyg. 1991;45:297–308.

10. WHO. Malaria Vaccine Technology Roadmap November 2013; [cited 23 Oct 2015]. Available from: http://www.who.int/immunization/topics/malaria/vaccine_roadmap/en/

11. Tuikue-Ndam N, Deloron P. Developing vaccines to prevent malaria in pregnant women. Expert Opin Biol Ther. 2015;15:1173–1182.

12. Mueller I, Shakri AR, Chitnis CE. Development of vaccines for Plasmodium vivax malaria. Vaccine. 2015 pii: S0264-410X(15)01336-5. doi:10.1016/j.vaccine.2015.09.060.

13. Morrison C. Landmark green light for Mosquirix malaria vaccine. Nat Biotechnol. 2015;33:1015–1016.

14. Crompton PD, Kayala MA, Traore B, et al. A prospective analysis of the Ab response to Plasmodium falciparum before and after a malaria season by protein microarray. Proc Natl Acad Sci U S A. 2010;107:6958–6963.

15. Malkin E, Hu J, Li Z, et al. A phase 1 trial of PFCP2.9: an AMA1/MSP1 chimeric recombinant protein vaccine for Plasmodium falciparum malaria. Vaccine. 2008;26:6864–6873.

16. Hu J, Chen Z, Gu J, et al. Safety and immunogenicity of a malaria vaccine, Plasmodium falciparum AMA-1/MSP-1 chimeric protein formulated in montanide ISA 720 in healthy adults. PLoS One. 2008;3:e1952.

17. Fowkes FJ, Richards JS, Simpson JA, et al. The relationship between anti-merozoite antibodies and incidence of Plasmodium falciparum malaria: a systematic review and meta-analysis. PLoS Med. 2010;7:e1000218.
This meta-analysis study has clearly shown the interpretation of immune correlates of protection in epidemiological studies is complicated.

18. Duncan CJ, Hill AV. Can growth inhibition assays (GIA) predict blood-stage malaria vaccine efficacy? Hum Vaccin Immunother. 2012;8:706–714.

19. Richards JS, Stanisic DJ, Fowkes FJ, et al. Association between naturally acquired antibodies to erythrocyte-binding antigens of Plasmodium falciparum and protection from malaria and high-density parasitemia. Clin Infect Dis. 2010;51:e50–60.

20. Rono J, Osier FH, Olsson D, et al. Breadth of anti-merozoite antibody responses is associated with the genetic diversity of asymptomatic Plasmodium falciparum infections and protection against clinical malaria. Clin Infect Dis. 2013;57:1409–1416.

21. Moss DK, Remarque EJ, Faber BW, et al. Plasmodium falciparum 19-kilodalton merozoite surface protein 1 (MSP1)-specific antibodies that interfere with parasite growth in vitro can inhibit MSP1 processing, merozoite invasion, and intracellular parasite development. Infect Immun. 2012;80:1280–1287.

22. Bergmann-Leitner ES, Duncan EH, Mullen GE, et al. Critical evaluation of different methods for measuring the functional activity of antibodies against malaria blood stage antigens. Am J Trop Med Hyg. 2006;75:437–442.

23. Malkin EM, Diemert DJ, McArthur JH, et al. Phase 1 clinical trial of apical membrane antigen 1: an asexual blood-stage vaccine for Plasmodium falciparum malaria. Infect Immun. 2005;73:3677–3685.

24. Polhemus ME, Magill AJ, Cummings JF, et al. Phase I dose escalation safety and immunogenicity trial of Plasmodium falciparum apical membrane protein (AMA-1) FMP2.1, adjuvanted with AS02A, in malaria-naïve adults at the Walter Reed Army Institute of Research. Vaccine. 2007;25:4203–4212.

25. Roestenberg M, Remarque E, De Jonge E, et al. Safety and immunogenicity of a recombinant Plasmodium falciparum AMA1 malaria vaccine adjuvanted with Alhydrogel, Montanide ISA 720 or AS02. PLoS One. 2008;3:e3960.

26. Ellis RD, Martin LB, Shaffer D, et al. Phase 1 trial of the Plasmodium falciparum blood stage vaccine MSP1(42)-C1/Alhydrogel with and without CPG 7909 in malaria naive adults. PLoS One. 2010;5:e8787.

27. El Sahly HM, Patel SM, Atmar RL, et al. Safety and immunogenicity of a recombinant nonglycosylated erythrocyte binding antigen 175 Region II malaria vaccine in healthy adults living in an area where malaria is not endemic. Clin Vaccine Immunol. 2010;17:1552–1559.

28. Dicko A, Diemert DJ, Sagara I, et al. Impact of a Plasmodium falciparum AMA1 vaccine on antibody responses in adult Malians. PLoS One. 2007;2:e1045.

29. Theria M, Dourboh OK, Coulibaly D, et al. Safety and immunogenicity of an AMA-1 malaria vaccine in Malian adults: results of a phase 1 randomized controlled trial. PLoS One. 2008;3:e1465.

30. Miura K, Zhou H, Diouf A, et al. Immunological responses against Plasmodium falciparum Apical Membrane Antigen 1 vaccines vary depending on the population immunized. Vaccine. 2011;29:2255–2261.

31. Miura K, Perera S, Brockley S, et al. Non-Apical Membrane Antigen 1 (AMA1) IgGs from Malian children interfere with functional activity of AMA1 IgGs as judged by growth inhibition assay. PLoS One. 2011;6:e20947.

32. Miura K, Zhou H, Moretz SE, et al. Comparison of biological activity of human anti-apical membrane antigen-1 antibodies induced by natural infection and vaccination. J Immunol. 2008;181:8776–8783.

33. Duncan CJ, Sheehy SH, Ewer KJ, et al. Impact on malaria parasite multiplication rates in infected volunteers of the protein-in-adjuvant vaccine AMA1-C1/Alhydrogel+CPG 7909. PLoS One. 2011;6:e22271.

34. Dutta S, Sullivan JS, Grady KK, et al. High antibody titer against apical membrane antigen-1 is required to protect against malaria in the Aotus model. PLoS One. 2009;4:e8138.

35. Singh S, Miura K, Zhou H, et al. Immunity to recombinant Plasmodium falciparum merozoite surface protein 1 (MSP1): protection in Aotus nancymai monkeys strongly correlates with anti-MSP1 antibody titer and in vitro parasite-inhibitory activity. Infect Immun. 2006;74:4573–4580.

36. Boyle MJ, Reiling L, Feng G, et al. Human antibodies fix complement to inhibit Plasmodium falciparum invasion of erythrocytes and are associated with protection against malaria. Immunity. 2015;42:580–590.

37. Boyle MJ, Wilson DW, Richards JS, et al. Isolation of viable Plasmodium falciparum merozoites to define erythrocyte invasion events and advance vaccine and drug development. Proc Natl Acad Sci U S A. 2010;107:14378–14383.

38. Bouharoun-Tayoun H, Oeuvray C, Lunel F, et al. Mechanisms underlying the monocyte-mediated antibody-dependent killing of Plasmodium falciparum asexual blood stages. J Exp Biol. 1995;182:409–418.

39. Bouharoun-Tayoun H, Attanath P, Sabchareon A, et al. Antibodies that protect humans against Plasmodium falciparum blood stage do not on their own inhibit parasite growth and invasion in vitro, but act in cooperation with monocytes. J Exp Med. 1990;172:1633–1641.

40. McCarthy JS, Marjason J, Elliott S, et al. A phase 1 trial of MSP2-C1, a blood-stage malaria vaccine containing 2 isoforms of MSP2 formulated with Montanide ISA 720. PLoS One. 2011;6:e24413.

41. Druilhe P, Spertini F, Soesoe D, et al. A malaria vaccine that elicits in humans antibodies able to kill Plasmodium falciparum. PLoS Med. 2005;2:e344.

42. Hermsen CC, Verhage DF, Telgt DS, et al. Glutamate-rich protein (GLURP) induces antibodies that inhibit in vitro growth of Plasmodium falciparum in a phase 1 malaria vaccine trial. Vaccine. 2007;25:2930–2940.

43. Yagi M, Bang G, Tougan T, et al. Protective epitopes of the Plasmodium falciparum SERAS5 malaria vaccine reside in intrinsically unstructured N-terminal repetitive sequences. PLoS One. 2014;9:e98460.

44. Chimma P, Roussillon C, Sratongno P, et al. A distinct peripheral blood monocyte phenotype is associated with parasite inhibitory activity in acute uncomplicated Plasmodium falciparum malaria. PLoS Pathog. 2009;5:e1000631.

45. Shi YP, Udhayakumar V, Oloo AJ, et al. Differential effect of functional activity of AMA1 IgGs on the growth of asexual stage
Plasmodium falciparum parasites. Am J Trop Med Hyg. 1999;60:135–141.

46. Tiendrebeogo RW, Adu B, Singh SK, et al. High-throughput tri-colour flow cytometry technique to assess Plasmodium falciparum parasitaemia in bioassays. Malar J. 2014;13:412.

47. Hill DL, Eriksson EM, Li Wai Suen CS, et al. Opsonising antibodies to P. falciparum merozoites associated with immunity to clinical malaria. PLoS One. 2013;8:e74627.

48. Osier FH, Feng G, Boyle MJ, et al. Opsonic phagocytosis of Plasmodium falciparum merozoites: mechanism in human immunity and a correlate of protection against malaria. BMC Med. 2014;12:108.

49. Ghumra A, Khunrae P, Ataide R, et al. Immunisation with recombinant PFEMP1 domains elicits functional rosette-inhibiting and phagocytosis-inducing antibodies to Plasmodium falciparum. PLoS One. 2011;6:e16414.

50. Chan JA, Howell KB, Reiling L, et al. Targets of antibodies against Plasmodium falciparum-infected erythrocytes in malaria immunity. J Clin Invest. 2012;122:3227–3238.

51. Zhou J, Feng G, Beeson J, et al. CD14(hi)CD16+ monocytes phagocyte antibody-opsonised Plasmodium falciparum infected erythrocytes more efficiently than other monocyte subsets, and require CD16 and complement to do so. BMC Med. 2015;13:154.

52. Fleit HB, Kobasiuk CD. The human monocyte-like cell line THP-1 expresses Fc gamma RI and Fc gamma RII. J Leukoc Biol. 1991;49:556–565.

53. Gilson PR, Crabbs BS. Morphology and kinetics of the three distinct phases of red blood cell invasion by Plasmodium falciparum merozoites. Int J Parasitol. 2009;39:91–96.

54. Boyle MJ, Wilson DW, Richards JS, et al. Isolation of viable Plasmodium falciparum merozoites to define erythrocyte invasion events and advance vaccine and drug development. Proc Natl Acad Sci USA. 2010;107:14378–14383.

55. Chan JA, Fowkes FJ, Beeson JG. Surface antigens of Plasmodium falciparum-infected erythrocytes as immune targets and malaria vaccine candidates. Cell Mol Life Sci. 2014;71:3633–3657.

56. Bengtsson A, Joergensen L, Rask TS, et al. A novel domain cassette identifies Plasmodium falciparum PFEMP1 proteins binding ICAM-1 and is a target of cross-reactive, adhesion-inhibitory antibodies. J Immunol. 2013;190:240–249.

57. Angeletti D, Albrecht L, Wahlgren M, et al. Analysis of antibody induction upon immunization with distinct NTS-DBL1alpha-domains of PFEMP1 from rosetting Plasmodium falciparum parasites. Malar J. 2013;12:32.

58. Guillotte M, Juillerat A, Igonet S, et al. Immunogenicity of the Plasmodium falciparum PFEMP1-VarO adhesin: induction of surface-reactive and rosette-disrupting antibodies to VarO infected erythrocytes. PLoS One. 2015;10:e0134292.

59. Hviid L, Jensen AT. PFEMP1 - a parasite protein family of key importance in Plasmodium falciparum malaria immunity and pathogenesis. Adv Parasitol. 2015;88:51–84.

60. Joos C, Marrama L, Polson HE, et al. Clinical protection from falciparum malaria correlations with neutrophil respiratory bursts induced by merozoites opsonized with human serum antibodies. PLoS One. 2010;5:e9871.

61. Llewellyn D, Miura K, Fay MP, et al. Standardization of the antibody-dependent respiratory burst assay with human neutrophils and Plasmodium falciparum malaria. Sci Rep. 2015;5:14081.

62. Baruch DI, Gamain B, Miller LH. DNA immunization with the cysteine-rich interdomain region 1 of the Plasmodium falciparum variant antigen elicits limited cross-reactive antibody responses. Infect Immun. 2003;71:4536–4543.

63. Gullingsrud J, Saveria T, Amos E, et al. Structure-function-immunogenicity studies of PFEMP1 domain DBL2betaPF11_0521, a malaria parasite ligand for ICAM-1. PLoS One. 2013;8:e61323.

64. Jeppesen A, Dittev SB, Soroka V, et al. Multiple Plasmodium falciparum erythrocyte membrane protein 1 (PFEMP1) variants per genome can bind IgM via Fcγ. Infect Immun. 2015;83:3972–3981.

65. Lennartz F, Bengtsson A, Olsen RW, et al. Mapping the binding site of a cross-reactive Plasmodium falciparum PFEMP1 monoclonal antibody inhibitory of ICAM-1 binding. J Immunol. 2015;195:3273–3283.

66. Ockenhouse CF, Angove E, Kester KE, et al. Phase I safety and immunogenicity trial of FMP1/AS02A, a Plasmodium falciparum MSP-1 asexual blood stage vaccine. Vaccine. 2006;24:3009–3017.

67. Nebie I, Diarra A, Ouedraogo A, et al. Humoral and cell-mediated immunity to MSP3 peptides in adults immunized with MSP3 in malaria endemic area, Burkina Faso. Parasite Immunol. 2009;31:474–480.

68. Spring MD, Cummings JF, Ockenhouse CF, et al. Phase 1/2a study of the malaria vaccine candidate apical membrane antigen-1 (AMA-1) administered in adjuvant system AS01B or AS02A. PLoS One. 2009;4:e5254.

69. Sheehy SH, Duncan CJ, Elias SC, et al. Phase I clinical evaluation of the safety and immunogenicity of the Plasmodium falciparum blood-stage antigen AMA1 in ChAd63 and MVA vaccine vectors. PLoS One. 2012;7:e31208.

70. Hodgson SH, Choudhary P, Elias SC, et al. Combining viral vectored and protein-in-adjuvant vaccines against the blood-stage malaria antigen AMA1 - report on a phase I clinical trial. Mol Ther. 2014;22:2142–2154.

71. Pombo DJ, Lawrence G, Hirunpetcharat C, et al. Immunity to malaria after administration of ultra-low doses of red cells infected with Plasmodium falciparum. Lancet. 2002;360:610–617.

72. Edstein MD, Kotecka BM, Anderson KL, et al. Lengthy antimalarial activity of atovaquone in human plasma following atovaquone-proguanil administration. Antimicrob Agents Chemother. 2005;49:4421–4422.

73. Collins WE, Walduck A, Sullivan JS, et al. Efficacy of vaccines containing rhoptry-associated proteins RAP1 and RAP2 of Plasmodium falciparum in Saimiri boliviensis monkeys. Am J Trop Med Hyg. 2000;62:466–479.

74. Stowers AW, Kennedy MC, Keegan BP, et al. Vaccination of monkeys with recombinant Plasmodium falciparum apical membrane antigen 1 confers protection against blood-stage malaria. Infect Immun. 2002;70:6961–6967.

75. Carvalho LJ, Oliveira SG, Theisen M, et al. Immunization of Saimiri sciureus monkeys with Plasmodium falciparum merozoite surface protein-3 and glutamate-rich protein
suggests that protection is related to antibody levels. Scand J Immunol. 2004;59:363–372.

76. Patarroyo ME, Alba MP, Curtidor H, et al. Using the PfEMP1 head structure binding motif to deal a blow at severe malaria. PLoS One. 2014;9:e88420.

77. Douglas AD, Williams AR, Knuepfer E, et al. Neutralization of Plasmodium falciparum merozoites by antibodies against PRfH. J Immunol. 2014;192:245–258.

78. Stowers AW, Miller LH. Are trials in New World monkeys on the critical path for blood-stage malaria vaccine development? Trends Parasitol. 2001;17:415–419.

79. Herrera S, Perlaza BL, Bonelo A, et al. Aotus monkeys: their great value for anti-malaria vaccines and drug testing. Int J Parasitol. 2002;32:1625–1635.

80. Sturchler D, Berger R, Rudin C, et al. Safety, immunogenicity, and pilot efficacy of Plasmodium falciparum sporozoite and asexual blood-stage combination vaccine in Swiss adults. Am J Trop Med Hyg. 1995;53:423–431.

81. Ockenhouse CF, Sun PF, Lanar DE, et al. Phase I/IIa safety, immunogenicity, and efficacy trial of NYVAC-PF7, a poxvected, multiantigen, multistage vaccine candidate for Plasmodium falciparum malaria. J Infect Dis. 1998;177:1664–1673.

82. Sedegah M, Tamminga C, McGrath S, et al. Adenovirus 5-vectored P. falciparum vaccine expressing CSP and AMA1. Part A: safety and immunogenicity in seronegative adults. PLoS One. 2011;6:e24586.

83. Thompson FM, Porter DW, Okitsu SL, et al. Evidence of blood stage efficacy with a virosomal malaria vaccine in a phase Ila clinical trial. PLoS One. 2008;3:e1493.

84. Tamminga C, Sedegah M, Maioletesi S, et al. Human adenovirus 5-vectored Plasmodium falciparum NMRC-M3V-Ad-PfCA vaccine encoding CSP and AMA1 is safe, well-tolerated and immunogenic but does not protect against controlled human malaria infection. Hum Vaccin Immunother. 2013;9:2165–2177.

85. Sheehy SH, Duncan CJ, Elias SC, et al. ChAd63-MVA-vectored blood-stage malaria vaccines targeting MSP1 and AMA1: assessment of efficacy against mosquito bite challenge in humans. Mol Ther. 2012;20:2355–2368.

86. Lawrence G, Cheng QQ, Reed C, et al. Effect of vaccination with 3 recombinant asexual-stage malaria antigens on initial growth rates of Plasmodium falciparum in non-immune volunteers. Vaccine. 2000;18:1925–1931.

87. Sauerwein RW, Roestenberg M, Moorthy VS. Experimental human challenge infections can accelerate clinical malaria vaccine development. Nat Rev Immunol. 2010;11:57–64.

88. Engwerda CR, Minigo G, Amante FH, et al. Experimentally induced blood stage malaria infection as a tool for clinical research. Trends Parasitol. 2012;28:515–521.

89. Sheehy SH, Douglas AD, Draper SJ. Challenges of assessing the clinical efficacy of asexual blood-stage Plasmodium falciparum malaria vaccines. Hum Vaccin Immunother. 2013;9:1831–1840.

90. Teirlinck AC, Roestenberg M, Van De Vegele-Bolmer M, et al. NF135.C10: a new Plasmodium falciparum clone for controlled human malaria infections. J Infect Dis. 2012;207:656–660.

91. Stanisic DJ, Liu XQ, De SL, et al. Development of cultured Plasmodium falciparum blood-stage malaria cell banks for early phase in vivo clinical trial assessment of anti-malaria drugs and vaccines. Malar J. 2015;14:143.

92. Siu E, Ploss A. Modeling malaria in humanized mice: opportunities and challenges. Ann N Y Acad Sci. 2015;1342:29–36.

93. Chen Q, Amaladoss A, Ye W, et al. Human natural killer cells control Plasmodium falciparum infection by eliminating infected red blood cells. Proc Natl Acad Sci U S A. 2014;111:1479–1484.

94. Wijayalath W, Majji S, Villasanes EF, et al. Humanized HLA-DR4.RagKO.II2RagmacKO.NOD (DRAG) mice sustain the complex vertebrate life cycle of Plasmodium falciparum malaria. Malar J. 2014;13:386.

95. Huang J, Li X, Coelho-Dos-Reis JG, et al. Human immune system mice immunized with Plasmodium falciparum circumsporozoite protein induce protective human humoral immunity against malaria. J Immunol Methods. 2015 pii: S0022-1759(15)30047-8. doi:10.1016/j.jim.2015.09.005.

96. Genton B, Betuela I, Felger I, et al. A recombinant blood-stage malaria vaccine reduces Plasmodium falciparum density and exerts selective pressure on parasite populations in a phase 1-2b trial in Papua New Guinea. J Infect Dis. 2002;185:820–827.

- The first phase II blood-stage vaccine trial which showed a strain-specific efficacy in the field.

97. Thera MA, Doumbo OK, Coulibaly D, et al. A field trial to assess a blood-stage malaria vaccine. N Engl J Med. 2011;365:1004–1013.

- The phase II blood-stage vaccine trial which showed a strain-specific protective efficacy in the field.

98. Sirima SB, Cousens S, Druilhe P. Protection against malaria by MSP3 candidate vaccine. N Engl J Med. 2011;365:1062–1064.

- A phase I blood-stage vaccine trial which showed a significant efficacy in the field.

99. Palacpac NM, Ntege E, Yeka A, et al. Phase 1b randomized trial and follow-up study in Uganda of the blood-stage malaria vaccine candidate BK-SE36. PLoS One. 2013;8:e64073.

- A phase I blood-stage vaccine trial which showed a significant efficacy in the field.

100. WHO. Malaria rainbow tables; [cited 2015 Oct 23]. Available from: http://www.who.int/immunization/research/development/Rainbow_tables/en/

101. ClinicalTrial.gov; [cited 2015 Oct 23]. Available from: https://clinicaltrials.gov/

102. Laurens MB, Thera MA, Coulibaly D, et al. Extended specific efficacy of a bivalent AMA1 malaria vaccine. Malar J. 2014. pii:10.1186/s12936-014-0052-7.

103. Sagara I, Dicko A, Ellis RD, et al. A randomized controlled phase 2 trial of the blood stage AMA1-C1/Alhydrogel malaria vaccine in children in Mali. Vaccine. 2010;28:5155–5160.

104. Ouattara A, Mu J, Takala-Harrison S, et al. Lack of allele-specific efficacy of a bivalent AMA1 malaria vaccine. Malar J. 2010;9:175.

105. Mullen GE, Ellis RD, Miura K, et al. Phase 1 trial of AMA1-C1/Alhydrogel plus CPG 7909: an asexual blood-stage
Dutta S, Dlugosz LS, Drew DR, et al. Overcoming antigenic diversity by enhancing the immunogenicity of conserved epitopes on the malaria vaccine candidate apical membrane antigen-1. PLoS Pathog. 2013;9:e1003840.

Miura K, Herrera R, Diouf A, et al. Overcoming allelic specificity by immunization with five allelic forms of Plasmodium falciparum apical membrane antigen 1. Infect Immun. 2013;81:1491–1501.

Kusi KA, Faber BW, Van Der Eijk M, et al. Immunization with different PFAMA1 alleles in sequence induces clonal imprint humoral responses that are similar to responses induced by the same alleles as a vaccine cocktail in rabbits. Malar J. 2011;10:40.

Kusi KA, Remarque EJ, Riasat V, et al. Safety and immunogenicity of multi-antigen AMA1-based vaccines formulated with CoVaccine HTTM and Montanide ISA 51 in rhesus macaques. Malar J. 2011;10:182.

Harris KS, Adda CG, Khore M, et al. Use of immunodamping to overcome diversity in the malarial vaccine candidate apical membrane antigen 1. Infect Immun. 2014;82:4707–4717.

Richard D, Macraild CA, Riglar DT, et al. 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. 2010;285:14815–14822.

Srinivasan P, Ekanem E, Diouf A, et al. Immunization with a functional protein complex required for erythrocyte invasion protects against lethal malaria. Proc Natl Acad Sci U S A. 2014;111:10311–10316.

Tsai CW, Duggan PF, Jin AJ, et al. Characterization of a protective Escherichia coli-expressed Plasmodium falciparum merozoite surface protein 3 indicates a non-linear, multi-domain structure. Mol Biochem Parasitol. 2009;164:45–56.

Audran R, Cachat M, Lurati F, et al. Phase I malaria vaccine trial with a long synthetic peptide derived from the merozoite surface protein 3 antigen. Infect Immun. 2005;73:8017–8026.

Tsai CW, Duggan PF, Jin AJ, et al. Characterization of a protective Escherichia coli-expressed Plasmodium falciparum merozoite surface protein 3 indicates a non-linear, multi-domain structure. Mol Biochem Parasitol. 2009;164:45–56.

Audran R, Cachat M, Lurati F, et al. Phase I malaria vaccine trial with a long synthetic peptide derived from the merozoite surface protein 3 antigen. Infect Immun. 2005;73:8017–8026.

Jeppsen MP, Jorgdand PS, Singh SK, et al. The malaria vaccine candidate GMZ2 elicits functional antibodies in individuals from malaria-endemic and non-endemic areas. J Infect Dis. 2013;208:479–488.

Esen M, Kremsner PG, Schleucher R, et al. Safety and immunogenicity of GMZ2 - a MSP3-GLURP fusion protein malaria vaccine candidate. Vaccine. 2009;27:6862–6868.

Mordmuller B, Szyvon K, Greutelaers B, et al. Safety and immunogenicity of the malaria vaccine candidate GMZ2 in malaria-exposed, adult individuals from Lambarene, Gabon. Vaccine. 2010;28:6698–6703.

Belard S, Issifou S, Hounkpatin AB, et al. A randomized controlled phase Ib trial of the malaria vaccine candidate GMZ2 in African children. PLoS One. 2011;6:e22525.

Hori T, Shirai H, Jie L, et al. Evidences of protection against blood-stage infection of Plasmodium falciparum by the novel protein vaccine SE36. Parasitol Int. 2010;59:380–386.

Darko CA, Angov E, Collins WE, et al. The clinical-grade 42-kilodalton fragment of merozoite surface protein 1 of Plasmodium falciparum strain FVO expressed in Escherichia coli protects Aotus nancymai against challenge with homologous erythrocytic-stage parasites. Infect Immun. 2005;73:287–297.

Lyon JA, Angov E, Fay MP, et al. Protection induced by Plasmodium falciparum MSP1(42) is strain-specific, antigen and adjuvant dependent, and correlates with antibody responses. PLoS One. 2008;3:e2830.

Ogutu BR, Apollo OJ, McKinney D, et al. Blood stage malaria vaccine eliciting high antigen-specific antibody concentrations confers no protection to young children in Western Kenya. PLoS One. 2009;4:e4708.

Healer J, Thompson JK, Riglar DT, et al. Vaccination with conserved regions of erythrocyte-binding antigens induces neutralizing antibodies against multiple strains of Plasmodium falciparum. PLoS One. 2013;8:e72504.

Bei AK, Membri CD, Rayner JC, et al. Variant merozoite protein expression is associated with erythrocyte invasion phenotypes in Plasmodium falciparum isolates from Tanzania. Mol Biochem Parasitol. 2007;153:66–71.

Villard V, Agak GW, Frank G, et al. Rapid identification of malaria vaccine candidates based on alpha-helical coiled coil protein motif. PLoS One. 2007;2:e645.

Kulangara C, Luedin S, Dietz O, et al. Cell biological characterization of the malaria vaccine candidate trophozoite exported protein 1. PLoS One. 2012;7:e46112.

Olugbile S, Kulangara C, Bang G, et al. Vaccine potentials of an intrinsically unstructured fragment derived from the blood stage associated P. falciparum protein PFF0165c. Infect Immun. 2009;77:5701–5709.

Douglas AD, Williams AR, Illingworth JH, et al. The blood-stage malaria antigen PRfH5 is susceptible to vaccine-inducible cross-strain neutralizing antibody. Nat Commun. 2011;2:601.

Tran TM, Onoiba A, Courens J, et al. Naturally acquired antibodies specific for Plasmodium falciparum reticulo- cyte-binding protein homologue 5 inhibit parasite growth and predict protection from malaria. J Infect Dis. 2014;209:789–798.

Patel SD, Ahouidi AD, Bei AK, et al. Plasmodium falciparum merozoite surface antigen, PRfH5, elicits detectable levels of invasion-inhibiting antibodies in humans. J Infect Dis. 2013;208:1679–1687.

Bustamante LY, Bartholdson SJ, Crosnier C, et al. A full-length recombinant Plasmodium falciparum PRfH5 protein induces inhibitory antibodies that are effective across common PRfH5 genetic variants. Vaccine. 2013;31:373–379.

Douglas AD, Baldeviano GC, Lucas CM, et al. A PRfH5-based vaccine is efficacious against heterologous strain blood-stage Plasmodium falciparum infection in Aotus monkeys. Cell Host Microbe. 2015;17:130–139.

Chen L, Lopatnicki S, Riglar DT, et al. An EGF-like protein forms a complex with PRfH5 and is required for invasion of human erythrocytes by Plasmodium falciparum. PLoS Pathog. 2011;7:e1002199.
135. Reddy KS, Amlb E, Pandey AK, et al. Multiprotein complex between the GPI-anchored CrRPA with PRH5 and PFRipr is crucial for Plasmodium falciparum erythrocyte invasion. Proc Natl Acad Sci U S A. 2015;112:1179–1184.

136. Williams AR, Douglas AD, Miura K, et al. Enhancing blockade of Plasmodium falciparum erythrocyte invasion: assessing combinations of antibodies against PRH5 and other merozoite antigens. PLoS Pathog. 2012;8:e1002991.

137. Reddy KS, Pandey AK, Singh H, et al. Bacterially expressed full-length recombinant Plasmodium falciparum RH5 protein binds erythrocytes and elicits potent strain-transcending parasite-neutralizing antibodies. Infect Immun. 2014;82:152–164.

138. Richards JS, Arumugam TU, Reiling L, et al. Identification and prioritization of merozoite antigens as targets of protective human immunity to Plasmodium falciparum malaria for vaccine and biomarker development. J Immunol. 2013;191:795–809.

139. Osier FH, Mackinnon MJ, Crosnier C, et al. New antigens for a multicomponent blood-stage malaria vaccine. Sci Transl Med. 2014;6:247ra102.

140. Raj DK, Nixon CP, Nixon CE, et al. Antibodies to PfSEA-1 block parasite egress from RBCs and protect against malaria infection. Science. 2014;344:871–877.

141. Ahlborg N, Iqbal J, Bjork L, et al. Plasmodium falciparum: differential parasite growth inhibition mediated by antibodies to the antigens Pf332 and Pf155/RESA. Exp Parasitol. 1996;82:155–163.

142. Balogun HA, Awah NW, Farouk SE, et al. Pf332-C231-reactive antibodies affect growth and development of intra-erythrocytic Plasmodium falciparum parasites. Vaccine. 2011;30:21–28.

143. De Silva HD, Saleh S, Kovacevic S, et al. The antibody response to Plasmodium falciparum merozoite surface protein 4: comparative assessment of specificity and growth inhibitory antibody activity to infection-acquired and immunization-induced epitopes. Malar J. 2011;10:266.

144. Zhao X, Chang Z, Tu Z, et al. PfRON3 is an erythrocyte-binding protein and a potential blood-stage vaccine candidate antigen. Malar J. 2014;13:490.

145. Arumugam TU, Takeo S, Yamasaki T, et al. Discovery of GAMA, a Plasmodium falciparum merozoite micronemal protein, as a novel blood-stage vaccine candidate antigen. Infect Immun. 2011;79:4523–4532.

146. Li X, Marinkovic M, Russo C, et al. Identification of a specific region of Plasmodium falciparum EBL-1 that binds to host receptor glycoporin B and inhibits merozoite invasion in human red blood cells. Mol Biochem Parasitol. 2012;183:23–31.

147. Sakamoto H, Takeo S, Maier AG, et al. Antibodies against a Plasmodium falciparum antigen PMSPDBL1 inhibit merozoite invasion into human erythrocytes. Vaccine. 2012;30:1972–1980.

148. Chiu CY, Hodder AN, Lin CS, et al. Antibodies to the Plasmodium falciparum proteins MSPDBL1 and MSPDBL2 opsonise merozoites, inhibit parasite growth and predict protection from clinical malaria. J Infect Dis. 2015;212:406–415.

149. Ito D, Hasegawa T, Miura K, et al. RALP1 is a rhoptry-neck erythrocyte-binding protein of Plasmodium falciparum merozoite and a potential blood-stage vaccine candidate antigen. Infect Immun. 2013;81:4290–4298.

150. Ellis RD, Wu Y, Martin LB, et al. Phase 1 study in malaria naïve adults of BSAM2/Alhydrogel(R)+CPG 7909, a blood stage vaccine against P. falciparum malaria. PLoS One. 2012;7:e46094.

151. Chitnis CE, Mukherjee P, Mehta S, et al. Phase I clinical trial of a recombinant blood stage vaccine candidate for Plasmodium falciparum malaria based on MSP1 and EBA175. PLoS One. 2015;10:e0117820.

152. Cech PG, Aebi T, Abdallah MS, et al. Virosome-formulated Plasmodium falciparum AMA-1 & CSP derived peptides as malaria vaccine: randomized phase 1b trial in semi-immune adults & children. PLoS One. 2011;6:e22273.

153. Theisen M, Roeffen W, Singh SK, et al. A multi-stage malaria vaccine candidate targeting both transmission and asexual parasite life-cycle stages. Vaccine. 2014;32:2623–2630.

154. Boes A, Spiegel H, Voepel N, et al. Analysis of a multi-component multi-stage malaria vaccine candidate tackling the cocktail challenge. PLoS One. 2015;10:e0131456.

155. Spiegel H, Boes A, Kastilan R, et al. The stage-specific in vitro efficacy of a malaria antigen cocktail provides valuable insights into the development of effective multistage vaccines. Biotechnol J. 2015;10:1651–1659.

156. Good MF, Reiman JM, Rodriguez IB, et al. Cross-species malaria immunity induced by chemically attenuated parasites. J Clin Invest. 2013;123:3353–3362.

157. Seder RA, Chang L-J, Enama ME, et al. Protection against malaria by intravenous immunization with a nonreplicating sporozoite vaccine. Science. 2013;341:1359–1365.

158. Bojang K, Milligan P, Pinder M, et al. Five year safety and immunogenicity of GlaxoSmithKline’s candidate malaria vaccine RTS,S/AS02 following administration to semi-immune adult men living in a malaria-endemic region of The Gambia. Hum Vaccin. 2009;5(4):242–247.

159. Kester KE, Cummings JF, Ofori-Anyinam O, et al. Randomized, double-blind, phase 2a trial of falciparum malaria vaccines RTS,S/AS01B and RTS,S/AS02A in malaria-naïve adults: safety, efficacy, and immunologic associates of protection. J Infect Dis. 2009;200:337–346.

160. Pierce MA, Ellis RD, Martin LB, et al. Phase 1 safety and immunogenicity trial of the Plasmodium falciparum blood-stage vaccine candidate AMA1-C1/ISA 720 in Australian adults. Vaccine. 2010;28:2236–2242.

161. Dutta S, Lee SY, Batchelor AH, et al. Structural basis of antigenic escape of a malaria vaccine candidate. Proc Natl Acad Sci U S A. 2007;104:12488–12493.

162. Wright KE, Hjerrild KA, Bartlett J, et al. Structure of malaria invasion protein RSH with erythrocyte basigin and blocking antibodies. Nature. 2014;515:427–430.

163. Ord RL, Caldeira JC, Rodriguez M, et al. A malaria vaccine candidate based on an epitope of the Plasmodium falciparum RSH protein. Malar J. 2014;13:326.

164. Dunachie S, Berthoud T, Hill AV, et al. Transcriptional changes induced by candidate malaria vaccines and correlation with protection against malaria in a human challenge model. Vaccine. 2015;33:5321–5331.