Chapter 14

Assessing Malaria Vaccine Efficacy

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Abstract

After many years of silence, eradication of malaria is, once again, one of the top priorities on the agenda of many international health and development agencies. To meet this idealistic goal, a combination of control tools is needed. From this armentarium, a malaria vaccine is central to prevent infection and/or disease. However, numerous malaria vaccine candidates have shown limited efficacy in Phase II and III studies. One reason for these failures has been that the assessment of efficacy in the context of malaria has been difficult to standardize. In this article, we have reviewed and discussed the different ways to assess the outcome of a malaria vaccination.

Keywords: malaria, vaccines, end-points, immunity, *Plasmodium*

1. Introduction

Malaria remains one of the major infectious diseases with a huge burden, affecting a large fraction of the world population. Although most of the deaths, caused by *Plasmodium falciparum*, *P. vivax* and to a lesser extent *P. malariae* and *P. ovale*, occurred in Africa; significant morbidity is evident in South America and Asia [1]. Different control measures such as insecticide-treated bed nets, powerful drugs (i.e., artemisinin-based combination therapies) and early diagnostics have had a positive impact in reducing malaria mortality worldwide [2]. However, these methods have led to complete eradication of malaria in only a few countries in intertropical zones [3]. This is mainly due to increasing drug resistance of the parasites and the failure of vector control strategies resulting from the change in mosquito behavior and the emergence of insecticide resistance [4, 5]. An antimalarial vaccine is thus a necessity to achieve the goal of complete global malaria eradication [6, 7].

Vaccine development in malaria have employed a composite of rational and empirical approaches and depended on multiple epidemiological and experimental studies. Individuals living in endemic regions acquire immunity over time after repeated exposure to the parasites. Such immunity, also called premonition, is partial, species-specific and biphasic [8, 9]. In the first phase, the hosts still get infected but do not develop clinical symptoms. On the contrary, the second phase, which is the prevention or limitation of parasite multiplication, takes long to develop. This second phase is heavily dependent on parasite exposure - more the exposure the host gets, lesser the time this immunity takes to develop [9, 10]. Hence, the goal of vaccine strategies is to reduce the time needed to acquire protective immunity and to make the immunity long-lasting.

The use of experimental models is critical to vaccine development. Many researchers advocate the use of human parasites in human hosts as it is the optimal experimental model for malaria [11]. However, field studies are inherently limited by the inability to control multiple experimental parameters such as the number of infective mosquito bites, the number of parasite per infective dose, and the genetic background of the host and parasite. In addition, there are numerous ethical considerations, which restrict access to peripheral blood samples for antibody and T cell studies, important for investigating long-term protection. Thus, many researchers have turned to more controllable models, such as monkey or human *Plasmodium* in monkeys [12–14] or rodent *Plasmodium* in mice [15]. Using these models, there have been numerous vaccination studies using genetically-attenuated parasites [16], irradiated parasites [17, 18], chemically-attenuated parasites [19, 20], live parasites under drug prophylaxis [21–23], and defined antigenic formulations [24–30]. These studies have demonstrated that vaccination can reduce parasite development, prevent pathology in infected animals, prevent transmission to mosquitoes, and even induce sterile immunity. Another major advantage of these models is that the outcomes following vaccination is well-defined and easily measurable, such as development of sterile immunity, inhibition of parasite development in the liver or in the blood, and/or prevention of certain pre-defined clinical signs or of pathologies. So far, only whole parasite formulations using irradiated sporozoites [31, 32] or live parasite immunization under chloroquine [33–35], and a limited number of sporozoite antigen formulations, such as RTS,S [36], have been shown to induce sterile immunity in significant proportion of the human volunteers.

2. Vaccine developmental phases

Vaccine clinical testing in humans involves multiple phases. Phase IA involves a small group of naïve volunteers (<100), from non-endemic regions, with no previous experience of malaria, while Phase I involves malaria-exposed individuals from endemic regions. In both phases, vaccine safety and immunogenicity are assessed. Only after the vaccine has shown a good safety profile with encouraging immunogenicity data, phase IIa test study can be initiated with a larger set of volunteers (>100–1000) from non-endemic regions. In Phase IIa, vaccine efficacy is assessed by subjecting the volunteers to a challenge with mosquito bites or intravenous injections of infected red blood cells. Phase IIb involves assessing the vaccine efficacy in a larger set of volunteers from endemic regions. Promising Phase II results qualify moving the

vaccine testing to Phase III, which comprises assessing vaccine safety (including potential side effects) and efficacy over a longer time period in a cohort consisting of thousands of volunteers from endemic regions. If sufficient safety and efficacy has been demonstrated in Phase III (2 to 5 years), the vaccine can then be licensed and marketed for human use, after which mass-deployment for endemic regions can be launched.

3. Testing malaria vaccines in the field

A malaria vaccine could potentially target many different stages of the infection. It could work by: (1) preventing *de novo* infection (either in the liver or the blood), (2) controlling parasite levels in the blood and duration of the blood infection, (3) preventing pathology induced by the infection and thus preventing or reducing morbidity or mortality, and (4) preventing or reducing transmission to mosquitoes. However, not all of these outcomes can be assessed accurately in the field. Only the first and fourth outcomes mentioned can be assessed accurately and experimentally with reproducible results, mainly due to the standard operating procedures that have been implemented over the years.

Outcome assessment in the field is complicated due to the nature of the infection itself. In endemic regions, malaria infections are usually chronic [37]. Low-level parasite persistence may affect immune reactivity by amplifying or down-regulating vaccine-induced immune responses. It may also confuse diagnostics such as fever detection [38]. Occurrence and extent of chronicity may vary according to age, endemicity and host genetics. Thus, in many trials, antimalarial treatments are applied to the tested cohorts to clear prior malarial infections to reduce confounding factors [39].

Presence of co-infections is another factor that makes outcome assessment in the field difficult. Often, endemic cohorts are also infected with other pathogens, such as worms, bacteria or viruses, without being overtly sick [40–44]. Immune responses to these pathogens may either potentiate or inhibit the development of the protective response induced by infection or vaccination [45]. Due to cost constraints, it is rarely possible to make a full analysis for all possible pathogens, but it is advisable to perform retrospective studies to assess their possible influence on the malaria vaccination outcomes.

Another major roadblock for malaria vaccine development is the absence of correlates or surrogate markers of protection. These markers are crucial as they would facilitate the testing of large sets of vaccine formulations and would reduce costs and organization constraints [46]. As an example, vaccine development against Hepatitis B was greatly simplified when it was shown that concentration of Hepatitis B S antigen antibodies over 10 UI/ML level was a surrogate marker of protection (for review Plotkin et al.) [47]. This greatly accelerated the testing of multiple new formulations in a limited number of volunteers and also helped in the development of subsequent improved formulations. There is clearly a gap in our knowledge of the immune correlates of protection against malaria. It is still not clearly known what defense mechanisms are crucial in humans for mediating protection against malaria. This severely handicaps our progress towards effective vaccine development [48].

To assess vaccine efficacy in the field, it is also critical to have epidemiological data concerning the vaccine site. The level of endemicity will have an impact on the surveillance time following the last immunization and also on the size of the cohort. Low endemic conditions will require longer follow-up and a larger cohort to obtain statistically significant results.

4. Vaccines targeting specific parasite stages

The malaria parasite has a complex life cycle, alternating between the human and mosquito host. In the human host, the malaria parasite transits across different body compartments and alternates between intracellular and extracellular locations (**Figure 1**). This developmental complexity of the malaria parasite has a profound impact on the study design of the malaria vaccine and assessment efficacy (**Table 1**).

During the pre-erythrocytic stage, the parasites exist as the extracellular motile sporozoite upon injection by the mosquito during feeding, and the intracellular liver parasites. Vaccines

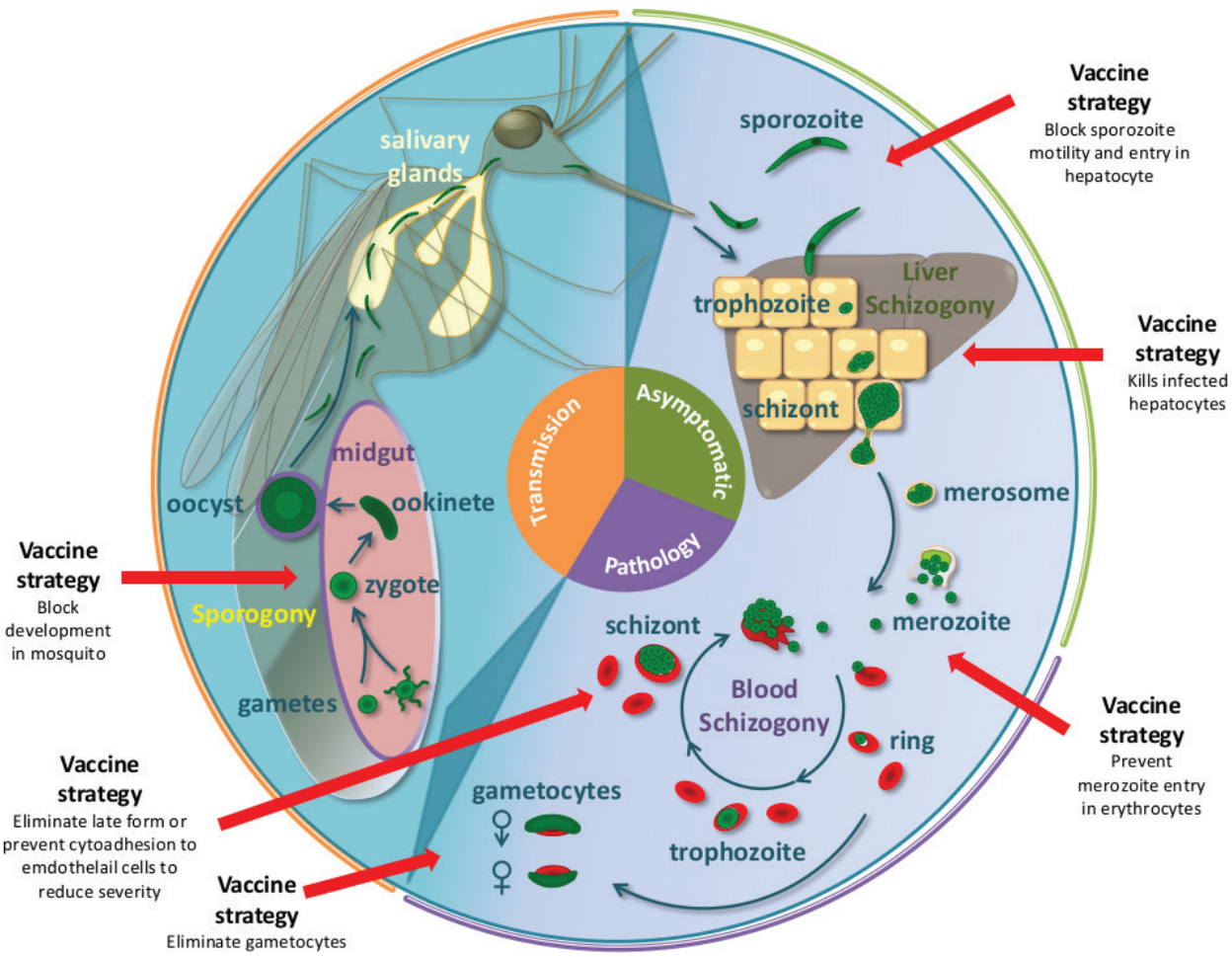

Figure 1. *Plasmodium falciparum* life cycle and vaccine strategies. The cycle in humans includes three stages: the pre-erythrocytic stage, which is asymptomatic; the asexual blood stage, which induces pathology; and the sexual stage, which is transmitted to *Anopheles* mosquitoes. At each of these stages, the parasite expresses various proteins that are targets of vaccine candidates. The different vaccine strategies for each stage are indicated.

Targets	Induced immunity	Mechanisms	Readout
Pre-erythrocytic vaccines			
Sporozoite antigens Liver stage antigens	Inhibition of parasite development and replication/ survival	Antibodies against sporozoites T cells against liver stage	Presence of parasites in the blood
Blood stage vaccines			
Asexual blood stage antigens	Inhibition of erythrocyte invasion and parasite replication/survival	Antibodies Antibody cell dependent inhibition (ADCI) Cellular immunity	Blood parasite load
Parasite derived toxins Parasite adhesion ligands	Inhibition of pathogenesis	Antibodies neutralizing inflammatory factors Antibodies inhibiting parasite/host interactions	Fever Blood parasite load Severe complications*
Transmission-blocking vaccines			
Sexual blood stage antigens Mosquito stage antigens	Inhibition of parasite development in the mosquito	Antibodies blocking gamete mating, ookinete formation or oocyst maturation	Presence of parasites in the mosquito vector (midgut, salivary glands)

Table 1. Targets and mechanisms for anti-malaria vaccines.

developed to target the pre-erythrocytic stage aim at inducing antibodies that target mainly the sporozoites and/or inducing T cells that will eliminate intracellular hepatic forms, thus preventing or controlling the extent of the subsequent blood stage development.

Vaccines targeting the asexual blood stages of the parasites are divided into two categories. The vaccines can be anti-parasite, which aim to control and eliminate parasite development in the blood or anti-disease, which aim to prevent the pathologies induced by the parasite. These vaccines need to induce different types of immune responses targeting different phases of the asexual blood stage. Vaccines targeting the sexual stage parasites aim to prevent transmission of gametocytes to the mosquito and/or gamete mating and ookinete development in the mosquito midgut.

It is worth noting that while the parasite expresses different set of genes at different stages of its life cycle, there are also many antigens that are expressed across the different parasite stages. Vaccination against these shared antigens may have an effect at different phases of the life cycle [22, 49–52], making them just as attractive for vaccine against malaria.

5. Anti-parasite vaccines

For anti-parasite vaccines targeting the pre-erythrocytic stage, the assessment of vaccine efficacy is relatively easy. Complete efficacy for this stage is defined as sterile protection, whereby

no parasite can be detected in blood of immunized individuals after the sporozoite challenge. This is an all or none phenomenon, because a single sporozoite developing in the liver can lead to full-blown blood infections.

Intuitively, one would expect great success of pre-erythrocytic vaccines since the limited numbers of sporozoites (a mean of 5–50) injected by infected mosquitoes [53] would be easily eliminated by the different arms of the immune system induced by the vaccine. However, this has proved to be the contrary. To date, only one vaccine formulation, RTS,S, an hybrid molecule containing a large segment of the circumsporozoite protein and S antigen of the Hepatitis B virus mixed with the AS02 adjuvant, has been shown to induce sterile protection in a substantial proportion of the naïve volunteers [36, 54] but to a much lower extent in field trials [55–58].

One reason that could contribute to the lack of success stories with pre-erythrocytic antiparasite vaccines is the procedures implemented to assess protection. Immunized volunteers were subjected to five mosquito bites, a dose required to ensure that naïve control volunteers would develop patent parasitemia 7–14 days after challenge [59, 60]. Alternative protocols using purified sporozoites injected either intradermal or intravenously have been developed, and so far, have proven to be safe and reproducible [61, 62]. Detection of parasitemia is performed by microscopy on Giemsa-stained blood smears over a 20–25-day period. Once a positive blood film is confirmed, the volunteers were treated with blood schizonticides to eliminate blood parasites and prevent any blood stage parasite-induced pathologies [63]. One limitation of this method of detection is that the time taken to detect parasites in the blood can differ up to 7 days. Hence, a delay of parasitemia does not necessarily translate in reduction of liver load. Moreover, there might be other confounding factors affecting the ability to detect blood parasitemia that are not related to the vaccination. To address this problem, sensitive PCR methods have been developed to detect the first wave of released liver merozoites and to assess the efficacy of the vaccine against pre-erythrocytic parasites. Using elegant regression methods, quantitative PCR techniques [64–67] allow an estimation of the reduction of the parasite liver load and an accurate measure of the effect on the growth rate of blood stage parasites.

Assessing pre-erythrocytic vaccine efficacy in the field is complicated due to factors mentioned earlier, such as the nature of the infection and presence of other co-infections. Evaluation of pre-erythrocytic vaccine in the field had mostly relied on microscopy and long follow-up (usually 6 to 24 months). As mentioned above, this assay may not be the most suitable to accurately assess the efficacy of any formulation targeting the pre-erythrocytic stage. In field conditions, many of the volunteers have been previously infected and, depending on age and exposure, may have developed some immunity against blood stage parasites. Thus, to eliminate possible confounding effects of a synergistic immunity of on-going blood stage infection with immunity induced by vaccination, it is important that volunteers are cleared by drug treatment of low-level parasitemia during immunization and before the surveillance period. It is also necessary that low-level blood infection occurrence be assessed by PCR. When implemented, this approach has reduced the follow-up time period to 1 month, saving costs and allowing the assessment of new formulations [39].

For vaccines against the pre-erythrocytic stage of *P. vivax*, efficacy assessment is further complicated by the fact that this species may produce non-replicating liver form called hypnozoites.

These hypnozoites are responsible for relapse up to 18 months after a sporozoite injection [68], thus complicating analysis and may require longer follow-up to detect relapse. Up to now, few challenges with *P. vivax* sporozoite have been performed [69]. There are no standard protocols and many issues need to be addressed [70]. First, the production of *P. vivax* sporozoites is limited since it requires infected blood from infected patients or monkeys to feed mosquitoes. Second, contrary to *P. falciparum*, no *P. vivax* cloned lines are available. Most of the lines available are derived from infected patients [71] or have been maintained in monkeys [72]. These lines contain multiple clones, which are poorly characterized at the molecular level [73]. This makes it difficult to obtain reproducible infection profile after experimental infection with mosquito bites of naïve volunteers and to characterize hypnozoite relapse profile. Moreover, as with anti-malarial drug studies in the field, the absence of validated genetic or serologic tools to distinguish between reinfection and relapse [74–77] may also prevent detecting strain-specific effect. For anti-parasite vaccines targeting the blood stage, efficacy is assessed after sporozoite or asexual blood stage parasite challenge. Sterile protection occurs when no parasite can be detected in blood of immunized individuals. Detection of parasitemia can be monitored either by microscopy or by PCR, the latter providing more information. Due to its higher sensitivity, it allows the detection of at least 3–5 parasite cycles even before the parasite is detectable by microscopy. PCR [78–81] bar-coding methods [82] can also be applied to genotype blood parasites. This allows assessing multiplicity of infection and determines whether the vaccine efficacy observed is strain-specific [83, 84]. Strain-specific vaccines have little interest since they will select vaccine-resistant parasites.

To assess the vaccine efficacy of anti-parasite vaccines, a challenge is essential. As mentioned earlier, challenge can be performed using sporozoites or blood stage parasites. However, due to the limited availability of insectaries that can provide infected mosquitoes on a regular basis, and the absence of accepted surrogates of protection, there is a necessity for blood stage challenge in healthy volunteers. Contrary to murine or monkey models where direct challenge with blood stage parasites is common, challenge with blood stage parasites in human has only been performed in limited vaccine studies using naïve volunteers [85, 86]. Because of safety reasons, blood parasites used for challenge need to be fully characterized. For a long time, only 3D7, a clone of the NF54 line, has been used. This line is susceptible to a wide range of antimalarials. Other parasite lines have been recently developed [87, 88]. However, since most of blood stage candidates are polymorphic, it is of utmost importance to assess the effect of polymorphism to have an idea of potential vaccine coverage. In addition, blood cells used for blood stage parasite propagation need to be pre-screened for the presence of a wide range of potential pathogens [86].

Despite these limitations, studies have shown that blood stage challenge can be safe and may allow the assessment of anti-asexual blood stage vaccine efficacy [89, 90]. Moreover, as recently reported, blood stage growth *in vivo* could be quantified more accurately after challenge with asexual blood stage parasites than with sporozoites [91], highlighting the advantage of this procedure. However, as it is not possible for safety reasons to let the parasitemia develop to high levels, its application might be limited. Vaccine efficacy may depend on the development of additional immune responses by the host during infection, which requires more time to be active as shown in mouse model [92]. In addition, some immune mechanisms

may need higher parasite challenge dose to be triggered. Antibody-dependent cell immunity or ADCI has been proposed to be effective with parasitemia approaching level detectable by microscopy [93].

In endemic settings, efficacy of blood stage vaccines has been assessed in natural conditions after challenge by mosquito bites. This type of challenge is perfectly suited if the vaccine can induce sterile immunity. However, it might not be the most appropriate when blood parasite multiplication rate must be measured. This rate depends on the numbers of liver merozoites released and timing of their release. As mentioned above, liver merozoite release is not a homogenous phenomenon in terms of quantity and timing. Thus, to obtain parasite growth curve suitable for comparative analysis between individuals and groups, blood sampling must be carefully planned. Indeed, this implies an active and close follow-up of the volunteers to obtain multiple time points. One possibility to obtain more homogenous results would be to perform the challenge with defined number of infected red blood cells at a same time of infection across all groups [86, 94]. However, this requires overcoming a series of hurdles such as the development of standardized inoculums with known number of parasites at the same stage of development and the availability of donor blood, which have to be heavily tested for the presence of any pathogens. In addition, the parasite in the challenge inoculums would also need to be fully characterized and clearly defined in term of parasite clonality.

To accurately assess the efficacy of anti-parasite blood stage vaccine, it is necessary to evaluate any pre-existing immune responses to the antigens in the blood stage vaccine. Individuals leaving in endemic areas acquire immunity over time. The time required to develop this immunity depends on the endemicity level and their genetic background. This immunity may influence growth rate of the parasite. Pre-existing immunity can synergize with the immunity induced by the vaccination. Vaccination may also boost pre-existing antigen-specific immune responses, which would be ideal for any vaccine formulations. On the contrary, pre-existing immune response may inhibit or mask the immune response induced by vaccination. It has been shown that the antibodies to the N-terminal of *P. falciparum* merozoite surface protein 1 can block the inhibitory activity of antibodies recognizing the C-terminal part [95]. Thus, if such an antibody interference mechanism exists for antigen(s) used in vaccine formulation, it would be necessary to evaluate carefully pre-existing immune responses to these antigens.

6. Anti-disease vaccines preventing or reducing morbidity and mortality

Anti-disease vaccines aim to prevent the pathologies induced by the parasite. Hence, to assess the efficacy of these vaccines, it is important to clearly define the symptoms. Symptomatic malaria infections are characterized by recurrent fever and if not treated could develop into more severe complications (i.e. anemia, multi-organ dysfunctions affecting the lungs, kidneys, liver and brain…), and ultimately leading to death. These different clinical occurrences can be considered as end-points when assessing vaccine efficacy. For safety and ethical reasons, these end-points are looked for in experimental clinical trials. However, they are not measured in

many field trials. Active and passive case detections are undertaken to detect clinical malaria episodes and define rate of the first episode or all episodes. Criteria to define a malaria case include presence of fever (≥37.5°C) and detection of malarial parasites in peripheral blood. Careful clinical assessment of the origin of fever is needed to ensure the fever is due to the parasite but not due to concomitant bacterial or viral infection. It should be mandatory to prevent undermining the vaccine efficacy. It is also crucial to clear any asymptomatic infections prior to vaccine testing. Clearing asymptomatic parasitemia allows a better identification of malaria-attributable fever [38]. Assessment of the reduction of severe symptom occurrence and mortality is more difficult to use as end-point. Because of active intervention (drug treatment and patient management), severity and mortality occur only in small fraction of clinical cases. Thus, in order to have sufficient statistical power to assess the vaccine testing, very large cohort is required, resulting in huge cost. Moreover, there have been concerns that decreasing the level of exposure to the parasites might, in return, results in an increase in mortality in the long–term [96]. It has been suggested that reduced exposure prevents the development of naturally-acquired clinical immunity [93], which is thought to result from constant parasite exposure. Thus, for any vaccines entering in Phase III trials, these end-points need to be assessed.

Two types of vaccine strategies aimed at reducing specifically morbidity and mortality are being developed. Anti-sequestration vaccines are based on the assumption that cytoadherence of infected red blood cells leading to parasite sequestration in deep tissues is responsible for most of malaria pathologies. These vaccines are designed to target parasite ligands such as members of the *var.* multigene family encoding the proteins *Plasmodium falciparum* erythrocyte membrane protein-1 (PfEMP-1), which mediates cytoadherence [97, 98]. It has been proposed that parasites sequester to avoid splenic elimination [99]. The more clinically-advanced anti-sequestration vaccine candidate aims at preventing pregnancy-associated malaria [100]. Few *var.* genes, which encode PfEMP-1 binding to chondroitin sulfate A (CSA), have been implicated in placental sequestration, thus making them attractive vaccine candidates [101, 102]. Anti-sequestration vaccines are designed to produce antibodies, which prevent the interactions between infected red blood cells and their cognate host cells (endothelial cells, syncytiotrophoblast…). This will lead to an increase in the circulation of blood parasites at all development stages and hence their elimination by the spleen. Primary end-point measures for such vaccines are both parasitological and clinical. Efficacy of desequestration as measured by the number of mature blood forms can be evaluated simply by microscopical observation of Giemsa stained-blood smears. However, preventing sequestration may lead to rapid increase of parasitemia and possibly a faster development of fever episodes and faster treatment application. Thus, the time-window for monitoring parasite development might be limited. Ultimately, assessment of severity and mortality are the most relevant measures for desequestration vaccines. A large cohort is needed to assess efficacy, like any blood stage vaccine targeting parasite growth.

Another type of anti-disease vaccine is targeting parasite moieties behaving as toxins and inducing immune-mediated pathologies [28, 103]. As these vaccines may have no effect on the parasitemia, the assessment of their efficacies will require very large cohorts to assess clinical outcomes with both active and passive case detection. In terms of safety, a strict clinical follow-up starting as early as the last vaccine dose administration is needed since these may perturb the immune network and induce immunopathology.

7. Transmission blocking vaccines

Transmission blocking vaccines (TBV) are designed to prevent or reduce the development of the sporogonic stage inside the mosquito host. This leads to a reduction in the numbers of infected mosquitoes and hence lesser malaria transmission in the population. As such, experimental clinical trials cannot provide straightforward answers of vaccine efficacy since the effect of such vaccine is at the population level and not at individual level. Assessment of TBV efficacy is done using *in vitro* assays and the membrane-feeding assay. Mosquitoes are fed *in vitro* with *Plasmodium* gametocytes mixed with serums from immunized individuals and the level of sporogonic development is assessed by counting the oocysts in dissected mosquito midguts [104] or, more rarely, the sporozoites in the mosquito salivary glands. Although this assay has been instrumental in identifying target antigens, it remains to be seen whether it might help to define correlate of protections for TBV development.

Currently, none of the TBV has progressed to clinical trials in the field. As the principal outcome of TBV is to reduce the number of infected mosquitoes at the population level, methods for assessing their number in field conditions should be implemented in a timely manner. There are, to date, no standardized methods to estimate the number of infected mosquitoes in the field and estimation would require large sampling size. Moreover, it seems that infected mosquitoes can cluster in discrete locations [105], thus requiring extensive studies on the distribution of infected mosquitos before and after vaccine trials. Since the number of infected mosquitoes depend on the number of circulating gametocytes [106], defining the number of gametocyte carrier prior to vaccine implementation is also a pre-requisite. In addition, defining transmission intensity of the vaccine site is important since it may influence the outcome of the vaccination. This can be defined by seroepidemiology and geographical information system (GIS) applications. For the latter, GPS mapping of mosquitoes and infected humans needs be done. It must be noted that one major limitation of these trials is that they assume that the human and mosquito populations tested are not mobile, which is often not true. An influx of infected individuals can modify the outcome by creating new reservoirs, and an influx of external infected mosquitoes would maintain transmission. It has been suggested that TBV can be tested with accuracy only in enclaved locations such as islands. Ultimately, the main expected outcome is that TBV will reduce transmission and thus reduce morbidity and mortality. The effect of such vaccine is at the population level, a large and costly cohort will be needed to be assessed over a long period of time. However, recent advances in modeling might facilitate TBV assessment by identifying end-point measures, which may serve as correlates of protection [107, 108]. Different end-point measures have been developed to assess TBV efficacy. For TBV that targets gametocytes, numbers of gametocytes and duration of gametocytes are important measures since it is expected that reduction in the number of gametocyte-carriers (reservoir) will decrease transmission. Microscopic determination of gametocytes on Giemsa-stained smears has long been used but they must be complemented with PCR methods since they have shown to underestimate gametocyte load [109]. Gametocyte infectivity to mosquitoes can be measured with the membrane-feeding assay mentioned earlier or with feeding of mosquitoes directly on the skin of gametocyte carriers, which reproduces the natural situation. An honest correlation

between the two assays has been described in few studies [110] but the membrane-feeding assay still awaits definitive validation [111]. In summary, it would be relevant for future TBV trials to perform feeding directly on gametocyte carriers using local mosquitoes. These mosquitoes would have to be raised in local insectaries and tested for the absence of any other human pathogens. Measures of TBV efficacy should not be limited to development of oocysts but also to salivary glands sporozoites since the latter are the infectious forms to humans. Hence, future studies should aim at measuring salivary gland sporozoite loads and sporozoite infectivity.

8. Concluding remarks

Here we discussed the different types of malaria vaccines and the different ways to access the vaccine efficacy. We also highlighted the limitations involved and the difficulties encountered by researchers aiming to develop an efficacious vaccine against a complex parasite such as *Plasmodium*. Despite decades of research efforts in vaccine development, no efficient malaria vaccine (i.e. with an efficacy >50%) has been developed. The most clinically-advanced RTS,S, which has been tested in Phase III, conferred at best 30–40% protection against clinical malaria [112]. Modeling studies have been proposed that, together with other malaria interventions, RTS,S vaccination may reduce the incidence of clinical malaria and deaths in many sub-Saharan African countries [113, 114]. Thus, this has led to the approval of licensure for the RTS,S vaccines by the European Union. However, the World Health Organization has not recommended its use in the extended program of immunization for children due to its discouraging vaccine efficacy data. Thus, the future of this vaccine for mass deployment remains uncertain. One of the major reasons of the limited efficacy of RTS,S vaccines and the discontinuation of various other vaccine development efforts is certainly due to antigen polymorphism [83]. In addition to antigen polymorphism, the malaria parasite utilizes many other immune escape mechanisms [115], which have severely hampered the development of malaria vaccines. With the renewed interest in malaria eradication, the development of an effective malaria vaccine is high on the agenda. Diverse strategies are being proposed to develop better vaccines: identification of new vaccine candidate [116], combinations of different antigens targeting the same stage or different stages [117]; new delivery systems and prime-boost strategies using different modalities [118]; and new adjuvants to induce stronger and longer lasting efficient immune responses [119–122]. However, for all vaccine types described, the absence of validated surrogates of protection to help select and prioritize different vaccine formulations is a major roadblock, which should be given priority to accelerate vaccine testing.

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References

[1] Gething PW, Hay SI, Lim SS. *Plasmodium falciparum* mortality in Africa between 1990 and 2015. New England Journal of Medicine. 2017;**376**:2494. DOI: 10.1056/NEJMc1701144

[2] Bhatt S, Weiss DJ, Cameron E, Bisanzio D, Mappin B, Dalrymple U, Battle KE, Moyes CL, Henry A, Eckhoff PA, Wenger EA, Briet O, Penny MA, Smith TA, Bennett A, Yukich J, Eisele TP, Griffin JT, Fergus CA, Lynch M, Lindgren F, Cohen JM, Murray CL, Smith DL, Hay SI, Cibulskis RE, Gething PW. The effect of malaria control on *Plasmodium falciparum* in Africa between 2000 and 2015. Nature. 2015;**526**:207-211. DOI: 10.1038/nature15535

[3] Rabinovich RN, Drakeley CJ, Djimde AA, Hall BF, Hay SI, Hemingway J, Kaslow DC, Noor A, Okumu F, Steketee R, Tanner M, Wells TNC, Whittaker MA, Winzeler EA, Wirth DF, Whitfield K, Alonso PL. MalERA: An updated research agenda for malaria elimination and eradication. PLoS Medicine. 2017;**14**:e1002456. DOI: 10.1371/journal.pmed.1002456

[4] Wiebe A, Longbottom J, Gleave K, Shearer FM, Sinka ME, Massey NC, Cameron E, Bhatt S, Gething PW, Hemingway J, Smith DL, Coleman M, Moyes CL. Geographical distributions of African malaria vector sibling species and evidence for insecticide resistance. Malaria Journal. 2017;**16**:85. DOI: 10.1186/s12936-017-1734-y

[5] Hemingway J, Ranson H, Magill A, Kolaczinski J, Fornadel C, Gimnig J, Coetzee M, Simard F, Roch DK, Hinzoumbe CK, Pickett J, Schellenberg D, Gething PW, Hoppe M, Hamon N. Averting a malaria disaster: will insecticide resistance derail malaria control? Lancet. 2016;**387**:1785-1788. DOI: 10.1016/S0140-6736(15)00417-1

[6] Malaria vaccine. WHO position paper – January 2016. Weekly Epidemiology Record. 2016; **4**:35-52

[7] Healer J, Cowman AF, Kaslow DC, Birkett AJ. Vaccines to accelerate malaria elimination and eventual eradication. Cold Spring Harbour Perspectives in Medicine. 2017;**7**:a025627. DOI: 10.1101/cshperspect.a025627

[8] Sergent E, Parrot L. L'Immunité, la prémunition et la resistance. Annales de l'institut Pasteur d'Algers. 1935;**13**:279-315

[9] McGregor IA. Malarial immunity: Current trends and prospects. Annals of Tropical Medicine and Parasitology. 1987;**81**:647-656

[10] Sinton JA. A summary of our present knowledge of the mechanism of immunity in malaria. Journal of Malaria Institute of India. 1939;**2**:71-83

[11] Druilhe P, Hagan P, Rook G. The importance of models of infection in the study of disease resistance. Trends in Microbiology. 2002;**10**:S38-S46

[12] Collins WE, Chin W, Skinner JC. *Plasmodium fragile* and *Macaca mulatta* monkeys as a model system for the study of malaria vaccines. American Journal of Tropical Medicine and Hygiene. 1979;**28**:948-954

[13] Gysin J. Relevance of the squirrel monkey as a model for experimental human malaria. Research in Immunology. 1991;**142**:649-654

[14] Heppner DG Jr, Cummings JF, Ockenhouse CF, Kester KE, Lyon JA, Gordon DM. New world monkey efficacy trials for malaria vaccine development: Critical path or detour? Trends in Parasitology. 2001;**17**:419-425

[15] Vanderberg JP. Reflections on early malaria vaccine studies, the first successful human malaria vaccination, and beyond. Vaccine. 2009;**27**:2-9. DOI: 10.1016/j.vaccine.2008.10.028

[16] Mueller AK, Labaied M, Kappe SH, Matuschewski K. Genetically modified *Plasmodium* parasites as a protective experimental malaria vaccine. Nature. 2005;**433**:164-167

[17] Richards WHG. Active immunization of chicks against *Plasmodium gallinaceum* by inactivated homologous sporozoites and erythrocytic parasites. Nature. 1966;**212**:1492-1494

[18] Nussenzweig RS, Vanderberg JP, Most H, Orton CG. Protective immunity produced by injection of X-irradiated sporozoites of *Plasmodium berghei*. Nature. 1967;**216**:160-162

[19] Purcell LA, Yanow SK, Lee M, Spithill TW, Rodriguez A. Chemical attenuation of *Plasmodium berghe*i sporozoites induces sterile immunity in mice. Infection and Immunity. 2008;**76**:1193-1887. DOI: 10.1128/IAI.01399-07

[20] Yanow SK, Purcell LA, Spithill TW. The a/T-specific DNA alkylating agent adozelesin inhibits *Plasmodium falciparum* growth in vitro and protects mice against *Plasmodium chabaudi adami* infection. Molecular Biochemical and Parasitology. 2006;**148**:52-59. DOI: 10.1128/IAI.01399-07

[21] Orjih AU, Cochrane AH, Nussenzweig RS. Comparative studies on the immunogenicity of infective and attenuated sporozoites of *Plasmodium berghei*. Transactions of the Royal Society of Tropical Medecine and Hygiene. 1982;**76**:57-61

[22] Belnoue E, Costa FTM, Frankenberg T, Vigario AM, Voza T, Leroy N, Rodrigues MM, Landau I, Snounou G, Renia L. Protective T cell immunity against malaria liver stage after vaccination with live sporozoites under chloroquine treatment. Journal of Immunology. 2004;**172**:2487-2495

[23] Friesen J, Silvie O, Putrianti ED, Hafalla JCR, Matuschewski K, Borrmann S. Natural immunization against malaria: Causal prophylaxis with antibiotics. Science Translational Medicine. 2010;**2**:40ra49. DOI: 10.1126/scitranslmed.3001058

[24] Zavala F, Tam JP, Barr PJ, Romero P, Ley V, Nussenzweig RS, Nussenzweig V. Synthetic peptide vaccine confers protection against murine malaria. Journal of Experimental Medicine. 1987;**166**:1591-1596

[25] Ling IT, Ogun SA, Holder AA. Immunization against malaria with a recombinant protein. Parasite Immunology. 1994;**16**:63-67

[26] Marussig M, Renia L, Motard A, Miltgen F, Pétour P, Chauhan VS, Corradin G, Mazier D. Linear and multiple antigen peptides containing defined T and B epitopes of the *Plasmodium yoelii* circumsporozoite protein: Antibody-mediated protection and boosting by sporozoite infection. International Immunology. 1997;**9**:1817-1824

[27] Miyahira Y, Garcia-Sastre A, Rodriguez D, Rodriguez JR, Murata K, Tsuji M, Palese P, Esteban M, Zavala F, Nussenzweig RS. Recombinant viruses expressing a human malaria antigen can elicit potentially protective immune CD8+ responses in mice. Proceedings of the National Academy of Sciences USA. 1998;**95**:3954-3959

[28] Schofield L, Hewitt MC, Evans K, Siomos MA, Seeberger PH. Synthetic GPI as a candidate anti-toxic vaccine in a model of malaria. Nature. 2002;**418**:785-789

[29] Draper SJ, Moore AC, Goodman AL, Long CA, Holder AA, Gilbert SC, Hill F, AVS H. Effective induction of high-titer antibodies by viral vector vaccines. Nature Medicine. 2008;**14**:819-821. DOI: 10.1038/nm.1850

[30] Li Y, Leneghan DB, Miura K, Nikolaeva D, Brian IJ, Dicks MD, Fyfe AJ, Zakutansky SE, de CS, Long CA, Draper SJ, AVS H, Hill F, Biswas S. Enhancing immunogenicity and transmission-blocking activity of malaria vaccines by fusing Pfs25 to IMX313 multimerization technology. Science Reports. 2016;**6**:18848. DOI: 10.1038/srep18848

[31] Hoffman SL, Goh LM, Luke TC, Schneider I, Le TP, Doolan DL, Sacci Jr JB, De la Vega P, Dowler M, Paul C, Gordon DM, Stoute JA, Church LW, Sedegah M, Heppner DG Jr, Ballou WR, Richie TL. Protection of humans against malaria by immunization with radiation-attenuated *Plasmodium falciparum* sporozoites. Journal of Infectious Diseases. 2002;**185**:1155-1164

[32] Seder RA, Chang LJ, Enama ME, Zephir KL, Sarwar UN, Gordon IJ, Holman LA, James ER, Billingsley PF, Gunasekera A, Richman A, Chakravarty S, Manoj A, Velmurugan S, Li M, Ruben AJ, Li T, Eappen AG, Stafford RE, Plummer SH, Hendel CS, Novik L, Costner PJ, Mendoza FH, Saunders JG, Nason MC, Richardson JH, Murphy J, Davidson SA, Richie TL, Sedegah M, Sutamihardja A, Fahle GA, Lyke KE, Laurens MB, Roederer MW, Tewari K, Epstein JE, Sim BK, Ledgerwood JE, Graham BS, Hoffman SL. Protection against malaria by intravenous immunization with a nonreplicating Sporozoite vaccine. Science. 2013;**341**:1359-1365. DOI: 10.1126/science.1241800

[33] Roestenberg M, McCall M, Hopman J, Wiersma J, Luty AJF, van Gemert GJ, van Der Vegte-Boklmer M, van Schaijk BCL, Teelen K, Arens T, Spaarman L, de Mast Q, Roeffen WFG, Snounou G, Renia L, van Der Ven AJAM, Hermsen CC, Sauerwein RW. Protection against a malaria challenge by sporozoite inoculation. New England Journal of Medicine. 2009;**361**:468-477

[34] Bijker EM, Schats R, Obiero JM, Behet MC, van Gemert GJ, Vegte-Bolmer MG, Graumans W, van Lieshout L, Bastiaens GJ, Teelen K, Hermsen CC, Scholzen A, Visser LG, Sauerwein RW. Sporozoite immunization of human volunteers under mefloquine prophylaxis is safe, immunogenic and protective: A double-blind randomized controlled clinical trial. PLoS One. 2014;**9**:e112910. DOI: 10.1371/journal.pone.0112910

[35] Mordmueller BG, Surat G, Lagler H, Chakravarty S, Ishizuka AS, Lalremruata A, Gmeiner M, Campo JJ, Esen M, Ruben AJ, Held J, Calle CL, Mengue JB, Gebru T, Ibanez J, Sulyok M, James ER, Billingsley PF, Natasha KC, Manoj A, Murshedkar T, Gunasekera A, Eappen AG, Li T, Stafford RE, Li M, Felgner PL, Seder RA, Richie TL, Sim BK, Hoffman SL, Kremsner PG. Sterile protection against human malaria by chemoattenuated PfSPZ vaccine. Nature. 2017;**542**:445-449. DOI: 10.1038/nature21060

[36] Kester KE, McKinney DA, Tornieporth N, Ockenhouse CF, Heppner DG Jr, Hall T, Krzych U, Delchambre M, Voss G, Dowler MG, Palensky J, Wittes J, Cohen J, Ballou WR. Efficacy of recombinant circumsporozoite protein vaccine regimens against experimental *Plasmodium falciparum* malaria. Journal of Infectious Diseases. 2001;**183**:640-647. DOI: 10.1086/318534

[37] Garnham PCC. Comments on biology of human malaria. Military Medicine. 1966;**131** (Suppl 2):131-132

[38] Bejon PA, Mwangi T, Lowe B, Peshu N, Hill AVS, Marsh K. Clearing asymptomatic parasitaemia increases the specificity of the definition of mild febrile malaria. Vaccine. 2007;**25**:8198-8202. DOI: 10.1016/j.vaccine.2007.07.057

[39] Imoukhuede EB, Andrews L, Milligan P, Berthoud T, Bojang KA, Nwakanma D, Ismaili J, Buckee CO, Njie F, Keita S, Sowe M, Lang T, Gilbert SC, Greenwood BM, Hill AVS. Low-level malaria infections detected by a sensitive polymerase chain reaction assay and use of this technique in the evaluation of malaria vaccines in an endemic area. American Journal of Tropical Medicine and Hygiene. 2007;**76**:486-493

[40] Menezes RAO, Gomes MDSM, Mendes AM, Couto AARA, Nacher M, Pimenta TS, Sousa ACP, Baptista ARS, Jesus MI, Enk MJ, Cunha MG, RLD M. Enteroparasite and vivax malaria co-infection on the Brazil-French Guiana border: Epidemiological, haematological and immunological aspects. PLoS One. 2018;**13**:e0189958. DOI: 10.1371/journal.pone.0190808

[41] Diallo MA, Kane BS, Ndiaye M, Dieng M, Diongue K, Badiane AS, Seck MC, Ndiaye D. *Plasmodium falciparum* malaria co-infection with tick-borne relapsing fever in Dakar. Malaria Journal. 2017;**16**:24. DOI: 10.1186/s12936-017-1682-6

[42] Shah PD, Mehta TK. Evaluation of concurrent malaria and dengue infections among febrile patients. Indian Journal of Medical Microbiology. 2017;**35**:402-405. DOI: 10.4103/ijmm.IJMM_15_455

[43] Waxman M, Aluisio AR, Rege S, Levine AC. Characteristics and survival of patients with Ebola virus infection, malaria, or both in Sierra Leone: A retrospective cohort study. Lancet Infectious Diseases. 2017;**17**:30112-30113. DOI: 10.1016/S1473-3099(17)30112-3

[44] Ayorinde AF, Oyeyiga AM, Nosegbe NO, Folarin OA. A survey of malaria and some arboviral infections among suspected febrile patients visiting a health Centre in Simawa, Ogun state, Nigeria. Journal of Infection and Public Health. 2016;**9**:52-59. DOI: 10.1016/j.jiph.2015.06.009

[45] Druilhe P, Tall A, Sokhna CS. Worms can worsen malaria: Towards a new means to roll back malaria? Trends in Parasitology. 2005;**21**:359-362. DOI: 10.1016/j.pt.2005.06.011

[46] Plotkin SA. Complex correlates of protection after vaccination. Clinical of Infectious Diseases. 2013;**56**:1458-1465

[47] Plotkin SA. Immunologic correlates of protection induced by vaccination. Pediatric Infectious Diseases Journal. 2001;**20**:63-75

[48] Dobano C, Campo JJ. Understanding protective immune mechanisms induced by malaria vaccines in the context of clinical trials. Human Vaccines. 2009;**5**:562-565

[49] Belnoue E, Voza T, Costa FTM, Gruner AC, Mauduit M, Rosa DS, Depinay N, Kayibanda M, Vigario AM, Mazier D, Snounou G, Sinnis P, Renia L. Vaccination with live *Plasmodium yoelii* blood stage parasites under chloroquine cover induces cross-stage immunity against malaria liver stage. Journal of Immunology. 2008;**181**:8552-8558

[50] Mazier D, Goma J, Pied S, Renia L, Nussler A, Miltgen F, Mattei DM, Grau GE. Hepatic phase of malaria: A crucial role as "go-between" with other stages. Bulletin of the World Health Organisation. 1990;**68**:126-131

[51] Mazier D, Renia L, Nussler A, Pied S, Marussig M, Goma J, Grillot D, Miltgen F, Drapier JC, Corradin G. Hepatic phase of malaria is the target of cellular mechanisms induced by the previous and the subsequent stages. A crucial role for liver nonparenchymal cells. Immunology Letters. 1990;**25**:65-70

[52] Motard A, Marussig M, Renia L, Baccam D, Landau I, Mattei DM, Targett G, Mazier D. Immunization with the malaria heat shock like protein hsp70-1 enhances transmission to the mosquito. International Immunology. 1995;**7**:147-150

[53] Beier JC, Davis JR, Vaughan JA, Noden BH, Beier MS. Quantitation of *Plasmodium falciparum* sporozoites transmitted *in vitro* by experimentally infected *Anopheles gambiae* and *Anopheles stephensi*. American Journal of Tropical Medicine and Hygiene. 1991;**44**:564-570

[54] Stoute JA, Slaoui M, Heppner DG Jr, Momin P, Kester KE, Desmons P, Wellde BT, Garcon N, Krzych U, Marchand M. A preliminary evaluation of a recombinant circumsporozoite protein vaccine against *Plasmodium falciparum* malaria. RTS,S Malaria Vaccine Evaluation Group. New England Journal of Medicine. 1997;**336**:86-91. DOI: 10.1056/NEJM199701093360202

[55] Bojang KA, Milligan PJM, Pinder M, Vigneron L, Alloueche A, Kester KE, Ballou WR, Conway DJ, Reece WH, Gothard PK, Yamuah LK, Delchambre M, Voss G, Greenwood BM, Hill AVS, McAdam KPWJ, Tornieporth N, Cohen JD, Doherty TM. Efficacy of RTS,S/AS02 malaria vaccine against *Plasmodium falciparum* infection in semi-immune

adult men in the Gambia: A randomised trial. Lancet. 2001;**358**:1927-1934. DOI: 10.1016/S0140-6736(01)06957-4

[56] Alonso PL, Sacarlal J, Aponte JJ, Leach A, Macete E, Milman J, Mandomando I, Spiessens B, Guinovart C, Espasa M, Bassat Q, Aide P, Ofori-Anyinam O, Navia MM, Corachan S, Ceuppens M, Dubois MC, Demoitie MA, Dubovsky F, Menendez C, Tornieporth N, Ballou WR, Thompson R, Cohen JD. Efficacy of the RTS,S/AS02A vaccine against *Plasmodium falciparum* infection and disease in young African children: Randomised controlled trial. Lancet. 2004;**364**:1411-1420

[57] Bejon PA, Lusingu J, Olotu AI, Leach A, Lievens M, Vekemans J, Mshamu S, Lang T, Gould J, Dubois MC, Demoitie MA, Stallaert JF, Vansadia P, Carter T, Njuguna P, Awuondo KO, Malabeja A, Abdul O, Gesase S, Mturi N, Drakeley CJ, Savarese B, Villafana T, Ballou WR, Cohen JD, Riley EM, Lemnge MM, Marsh K, von Seidlein LV: Efficacy of RTS,S/AS01E vaccine against malaria in children 5 to 17 months of age. New England Journal of Medicine. 2008;**359**:2521-2532. DOI: 10.1016/S0140-6736(04)17223-1

[58] RTS,S Clinical Trials Partnership, Agnandji ST, Lell B, Soulanoudjingar SS, Fernandes JF, Abossolo BP, Conzelmann C, Methogo BG, Doucka Y, Flamen A, Mordmueller BG, Issifou S, Kremsner PG, Sacarlal J, Aide P, Lanaspa M, Aponte JJ, Nhamuave A, Quelhas D, Bassat Q, Mandjate S, Macete E, Alonso P, Abdulla S, Salim N, Juma O, Shomari M, Shubis K, Machera F, Hamad AS, Minja R, Mtoro A, Sykes A, Ahmed S, Urassa AM, Ali AM, Mwangoka G, Tanner M, Tinto H, D'Alessandro U, Sorgho H, Valea I, Tahita MC, Kabore W, Ouedraogo S, Sandrine Y, Guiguemde RT, Ouedraogo JB, Hamel MJ, Kariuki S, Odero C, Oneko M, Otieno K, Awino N, Omoto J, Williamson J, Muturi-Kioi V, Laserson KF, Slutsker L, Otieno W, Otieno L, Nekoye O, Gondi S, Otieno A, Ogutu B, Wasuna R, Owira V, Jones D, Onyango AA, Njuguna P, Chilengi R, Akoo P, Kerubo C, Gitaka J, Maingi C, Lang T, Olotu A, Tsofa B, Bejon PA, Peshu N, Marsh K, Owusu-Agyei S, Asante KP, Osei-Kwakye K, Boahen O, Ayamba S, Kayan K, Owusu-Ofori R, Dosoo D, Asante I, Adjei G, Adjei G, Chandramohan D, Greenwood BM, Lusingu J, Gesase S, Malabeja A, Abdul O, Kilavo H, Mahende C, Liheluka E, Lemnge M, Theander TG, Drakeley CJ, Ansong D, Agbenyega T, Adjei S, Boateng HO, Rettig T, Bawa J, Sylverken J, Sambian D, Agyekum A, Owusu L, Martinson F, Hoffman I, Mvalo T, Kamthunzi P, Nkomo R, Msika A, Jumbe A, Chome N, Nyakuipa D, Chintedza J, Ballou WR, Bruls M, Cohen J, Guerra Y, Jongert E, Lapierre D, Leach A, Lievens M, Ofori-Anyinam O, Vekemans J, Carter T, Leboulleux D, Loucq C, Radford A, Savarese B, Schellenberg D, Sillman M, Vansadia P. First results of phase 3 trial of RTS,S/AS01 malaria vaccine in African children. New England Journal of Medicine. 2011;**365**:1863-1875. DOI: 10.1056/NEJMoa1102287

[59] Roestenberg M, Mordmueller BG, Ockenhouse CF, Mo A, Yazdanbakhsh M, Kremsner PG. The frontline of controlled human malaria infections: A report from the controlled human infection models workshop in Leiden University Medical Centre 5 May 2016. Vaccine. 2017;**35**:7065-7069. DOI: 10.1016/j.vaccine.2017.10.092

[60] Roestenberg M, O'Hara GA, Duncan CJ, Epstein JE, Edwards NJ, Scholzen A, van Der Ven AJAM, Hermsen CC, Hill AVS, Sauerwein RW. Comparison of clinical and parasitological

data from controlled human malaria infection trials. PLoS One. 2012;**7**:e38434. DOI: 10.1371/journal.pone.0038434

[61] Roestenberg M, Bijker EM, Sim BK, Billingsley PF, James ER, Bastiaens GJ, Teirlinck AC, Scholzen A, Teelen K, Arens T, van der Ven AJ, Gunasekera A, Chakravarty S, Velmurugan S, Hermsen CC, Sauerwein RW, Hoffman SL. Controlled human malaria infections by intradermal injection of cryopreserved *Plasmodium falciparum* sporozoites. American Journal of Tropical Medicine and Hygiene. 2013;**88**:5-13. DOI: 10.4269/ajtmh.2012.12-0613

[62] Sissoko MS, Healy SA, Katile A, Omaswa F, Zaidi I, Gabriel EE, Kamate B, Samake Y, Guindo MA, Dolo A, Niangaly A, Niare K, Zeguime A, Sissoko K, Diallo H, Thera I, Ding K, Fay MP, O'Connell EM, Nutman TB, Wong-Madden S, Murshedkar T, Ruben AJ, Li M, Abebe Y, Manoj A, Gunasekera A, Chakravarty S, Sim BK, Billingsley PF, James ER, Walther M, Richie TL, Hoffman SL, Doumbo O, Duffy PE. Safety and efficacy of PfSPZ vaccine against *Plasmodium falciparum* via direct venous inoculation in healthy malaria-exposed adults in Mali: A randomised, double-blind phase 1 trial. Lancet Infectious Diseases. 2017;**17**:498-509. DOI: 10.1016/S1473-3099(17)30104-4

[63] Sauerwein RW, Roestenberg M, Moorthy VS. Experimental human challenge infections can accelerate clinical malaria vaccine development. Nature Reviews. Immunology. 2011;**11**:57-64. DOI: 10.1038/nri2902

[64] Hermsen CC, De Vlas SJ, van Gemert GJ, Telgt DS, Verhage DF, Sauerwein RW. Testing vaccines in human experimental malaria: Statistical analysis of parasitemia measured by a quantitative real-time polymerase chain reaction. American Journal of Tropical Medicine and Hygiene. 2004;**71**:196-201

[65] Andrews L, Andersen RF, Webster D, Dunachie S, Walther RM, Bejon PA, Hunt-Cooke A, Bergson G, Sanderson F, Hill AVS, Gilbert SC. Quantitative real-time polymerase chain reaction for malaria diagnosis and its use in malaria vaccine clinical trials. American Journal of Tropical Medicine and Hygiene. 2005;**73**:191-198

[66] Bejon PA, Andrews L, Andersen RF, Dunachie S, Webster D, Walther M, Gilbert SC, Peto TEA, Hill AVS. Calculation of liver-to-blood Inocula, parasite growth rates, and Preerythrocytic vaccine efficacy, from serial quantitative polymerase chain reaction studies of volunteers challenged with malaria Sporozoites. Journal of Infectious Diseases. 2005;**191**:619-626. DOI: 10.1086/427243

[67] Roestenberg M, De Vlas SJ, Nieman AE, Sauerwein RW, Hermsen CC: Efficacy of Preerythrocytic and blood-stage malaria vaccines can be assessed in small sporozoite challenge trials in human volunteers. Journal of Infectious Diseases. 2012;**206**:319-323. DOI: 10.1093/infdis/jis355

[68] Coatney GR, Cooper WC, Young MD. Studies on human malaria. XXX. A summary of 204 sporozoite-induced infections with the Chesson strain of *Plasmodium vivax*. Journal of the National Malaria Society. 1951;**10**:381-396

[69] Arevalo-Herrera M, Forero-Pena DA, Rubiano K, Gomez-Hincapie J, Martinez NL, Lopez-Perez M, Castellanos A, Cespedes N, Palacios R, Onate JM, Herrera S. *Plasmodium vivax* sporozoite challenge in malaria-naive and semi-immune colombian volunteers. PLoS One. 2014;**9**:e99754. DOI: 10.1371/journal.pone.0099754

[70] Mueller I, Moorthy VS, Brown GV, Smith PG, Alonso PL, Genton B. Guidance on the evaluation of *Plasmodium vivax* vaccines in populations exposed to natural infection. Vaccine. 2009;**27**:5633-5643. DOI: 10.1016/j.vaccine.2009.07.018

[71] Payne RO, Griffin PM, McCarthy JS, Draper SJ. *Plasmodium vivax* controlled human malaria infection - progress and prospects. Trends in Parasitology. 2017;**33**:141-150. DOI: 10.1016/j.pt.2016.11.001

[72] Collins WE, Sullivan JS, Strobert E, Galland GG, Williams A, Nace D, Williams T, Barnwell JW. Studies on the Salvador I strain of *Plasmodium vivax* in non-human primates and anopheline mosquitoes. American Journal of Tropical Medicine and Hygiene. 2009, 2009;**80**:228-235

[73] Ntumngia FB, McHenry AM, Barnwell JW, Cole-Tobian JL, King CL, Adams JH. Genetic variation among *Plasmodium vivax* isolates adapted to non-human primates and the implication for vaccine development. American Journal of Tropical Medicine and Hygiene. 2009;**80**:218-227

[74] Imwong M, Snounou G, Pukrittayakamee S, Tanomsing N, Kim JR, Nandy A, Guthmann JP, Nosten F, Carlton JMR, Looareesuwan S, Nair S, Sudimack D, Day NP, Anderson TJC, White NJ. Relapses of *Plasmodium vivax* infection usually result from activation of heterologous Hypnozoites. Journal of Infectious Diseases. 2007;**195**:927-933. DOI: 10.1086/512241

[75] Imwong M, Boel ME, Pagornrat W, Pimanpanarak M, McGready RM, Day NP, Nosten F, White NJ. The first *Plasmodium vivax* relapses of life are usually genetically homologous. Journal of Infectious Diseases. 2012;**205**:860-863. DOI: 10.1093/infdis/jir806

[76] Lin JT, Patel JC, Kharabora O, Sattabongkot J, Muth S, Ubalee R, Schuster AL, Rogers WO, Wongsrichanalai C, Juliano JJ. *Plasmodium vivax* isolates from Cambodia and Thailand show high genetic complexity and distinct patterns of *P. vivax* multidrug resistance gene 1 (pvmdr1) polymorphisms. American Journal of Tropical Medicine and Hygiene. 2013;**88**:1116-1123. DOI: 10.4269/ajtmh.12-0701

[77] Chuquiyauri R, Molina DM, Moss EL, Wang R, Gardner MJ, Brouwer KC, Torres S, Gilman RH, Llanos-Cuentas A, Neafsey DE, Felgner PL, Liang X, Vinetz JM. Genome-scale protein microarray comparison of human antibody responses in *Plasmodium vivax* relapse and reinfection. American Journal of Tropical Medicine and Hygiene. 2015;**93**:801-809. DOI: 10.4269/ajtmh.15-0232

[78] Snounou G. Genotyping of *Plasmodium* spp. Nested PCR. Malaria Methods and Protocols. 2002:103-116. DOI: 10.1385/1-59259-271-6:189

[79] Perandin F, Manca N, Calderaro A, Piccolo G, Galati L, Ricci L, Medici MC, Arcangeletti MC, Snounou G, Dettori G, Chezzi C. Development of a real-time PCR assay for detection of *Plasmodium falciparum*, *Plasmodium vivax,* and *Plasmodium ovale* for routine clinical diagnosis. Journal of Clinical Microbiology. 2004;**42**:1214-1219

[80] Mugittu K, Adjuik M, Snounou G, Ntoumi F, Taylor W, Mshinda H, Olliaro P, Beck HP. Molecular genotyping to distinguish between recrudescents and new infections in treatment trials of *Plasmodium falciparum* malaria conducted in sub-Saharan Africa: Adjustment of parasitological outcomes and assessment of genotyping effectiveness. Tropical Medicine and Intrnational Health. 2006;**11**:1350-1359. DOI: 10.1111/j.1365-3156.2006.01688.x

[81] Imwong M, Hanchana S, Malleret B, Renia L, Day NP, Dondorp AM, Nosten F, Snounou G, White NJ. High throughput ultra-sensitive molecular techniques to quantify low density malaria parasitaemias. Journal of Clinical Microbiology. 2014;**52**:3303-3309. DOI: 10.1128/JCM.01057-14

[82] Preston MD, Campino SG, Assefa SA, Echeverry DF, Ocholla H, Amambua-Ngwa A, Stewart LB, Conway DJ, Borrmann S, Michon P, Zongo I, Ouedraogo JB, Djimde AA, Doumbo OK, Nosten F, Pain A, Bousema JT, Drakeley CJ, Fairhurst RM, Sutherland CJ, Roper C, Clark TG. A barcode of organellar genome polymorphisms identifies the geographic origin of *Plasmodium falciparum* strains. Nature Communications. 2014;**5**:4052. DOI: 10.1038/ncomms5052

[83] Neafsey DE, Juraska M, Bedford T, Benkeser D, Valim C, Griggs A, Lievens M, Abdulla S, Adjei S, Agbenyega T, Agnandji ST, Aide P, Anderson S, Ansong D, Aponte JJ, Asante KP, Bejon PA, Birkett AJ, Bruls M, Connolly KM, D'Alessandro U, Dobano C, Gesase S, Greenwood BM, Grimsby J, Tinto H, Hamel MJ, Hoffman I, Kamthunzi P, Kariuki S, Kremsner PG, Leach A, Lell B, Lennon NJ, Lusingu J, Marsh K, Martinson F, Molel JT, Moss EL, Njuguna P, Ockenhouse CF, Ragama OB, Otieno W, Otieno L, Otieno K, Owusu-Agyei S, Park DJ, Pelle K, Robbins D, Russ C, Ryan EM, Sacarlal J, Sogoloff B, Sorgho H, Tanner M, Theander TG, Valea I, Volkman SK, Yu Q, Lapierre D, Birren BW, Gilbert PB, Wirth DF. Genetic diversity and protective efficacy of the RTS,S/AS01 malaria vaccine. New England Journal of Medicine. 2015;**373**:2025-2037. DOI: 10.1056/NEJMoa1505819

[84] Thera MA, Doumbo OK, Coulibaly D, Laurens MB, Ouattara A, Kone AK, Guindo AB, Traore K, Traore I, Kouriba B, Diallo DA, Diarra I, Daou M, Dolo A, Tolo Y, Sissoko MS, Niangaly A, Sissoko M, Takala-Harrison SL, Lyke KE, Wu Y, Blackwelder WC, Godeaux O, Vekemans J, Dubois MC, Ballou WR, Cohen JD, Thompson D, Dube T, Soisson L, Diggs CL, House B, Lanar DE, Dutta S, Heppner Jr DG, Plowe CV. A field trial to assess a blood-stage malaria vaccine. New England Journal of Medicine. 2011;**365**:1004-1013. DOI: 10.1056/NEJMoa1008115

[85] Duncan CJA, Draper SJ. Controlled human blood stage malaria infection: Current status and potential applications. American Journal of Tropical Medicine and Hygiene. 2012;**86**:561-565. DOI: 10.1056/NEJMoa1008115

[86] Stanisic DI, McCarthy JS, Good MF. Controlled human malaria infection: Applications, advances, and challenges. Infection and Immunity. 2018;**86**:e00479017. DOI: 10.1128/IAI.00479-17

[87] Teirlinck AC, Roestenberg M, van Der Ven AJAM, Scholzen A, Heinrichs MJ, Siebelink-Stoter R, Graumans W, van Gemert GJ, Teelen K, Vos MW, Nganou-Makamdop K, Borrmann S, Rozier YP, Erkens MA, Luty AJF, Hermsen CC, Sim BK, van Lieshout AW, Hoffman SL, Visser LG, Sauerwein RW. NF135.C10: A new *Plasmodium falciparum* clone for controlled human malaria infections. Journal of Infectious Diseases. 2013;**207**:656-666. DOI: 10.1093/infdis/jis725

[88] Lyke KE, Ishizuka AS, Berry AA, Chakravarty S, DeZure A, Enama ME, James ER, Billingsley PF, Gunasekera A, Manoj A, Li M, Ruben AJ, Li T, Eappen AG, Stafford RE, Kc N, Murshedkar T, Mendoza FH, Gordon IJ, Zephir KL, Holman LA, Plummer SH, Hendel CS, Novik L, Costner PJ, Saunders JG, Berkowitz NM, Flynn BJ, Nason MC, Garver LS, Laurens MB, Plowe CV, Richie TL, Graham BS, Roederer M, Sim BK, Ledgerwood JE, Hoffman SL, Seder RA. Attenuated PfSPZ vaccine induces strain-transcending T cells and durable protection against heterologous controlled human malaria infection. Proceedings of the National Academy of Sciences of USA. 2017;**114**:2711-2716. DOI: 10.1073/pnas.1615324114

[89] Lawrence GW, Cheng Q, Reed C, Taylor D, Stowers AW, Cloonan N, Rzepczyk CM, Smillie A, Anderson K, Pombo DJ, Allworth A, Eisen D, Anders RF, Saul AJ. 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

[90] Pombo DJ, Lawrence G, Hirunpetcharat C, Rzepczyk CM, Bryden M, Cloonan N, Anderson K, Mahakunkijcharoen Y, Martin LB, Wilson D, Elliot S, Eisen DP, Weinberg JB, Saul AJ, Good MF. Immunity to malaria after administration of ultra-low doses of red cells infected with *Plasmodium falciparum*. Lancet. 2002;**360**:610-617. DOI: 10.1016/S0140-6736(02)09784-2

[91] Sanderson F, Andrews L, Douglas AD, Hunt-Cooke A, Bejon PA, Hill AVS. Blood-stage challenge for malaria vaccine efficacy trials: A pilot study with discussion of safety and potential value. American Journal of Tropical Medicine and Hygiene. 2008;**78**:878-883

[92] Hirunpetcharat C, Vukovic P, Liu XQ, Kaslow DC, Miller LH, Good MF. Absolute requirement for an active immune response involving B cells and Th cells in immunity to *Plasmodium yoelii* passively acquired with antibodies to the 19-kDa carboxyl-terminal fragment of merozoite surface protein-1. Journal of Immunology. 1999;**162**:7309-7314

[93] Druilhe P, Perignon JL. A hypothesis about the chronicity of malaria infection. Parasitology Today. 1997;**13**:353-357

[94] Griffin P, Pasay C, Elliott S, Sekuloski S, Sikulu M, Hugo L, Khoury D, Cromer D, Davenport M, Sattabongkot J, Ivinson K, Ockenhouse CF, McCarthy J. Safety and reproducibility of a clinical trial system using induced blood stage *Plasmodium vivax* infection and its potential as a model to evaluate malaria transmission. PLoS Neglected Tropical Diseases. 2016;**10**:e0005139. DOI: 10.1371/journal.pntd.00051390

[95] Guevara Patino JA, Holder AA, McBride JS, Blackman MJ. Antibodies that inhibit malaria merozoite surface protein-1 processing and erythrocyte invasion are blocked by naturally acquired human antibodies. Journal of Experimental Medicine. 1997;**186**:**1**689-1699

[96] Ghani AC, Sutherland CJ, Riley EM, Drakeley CJ, Griffin JT, Gosling RD, Filipe JA. Loss of population levels of immunity to malaria as a result of exposure-reducing interventions: Consequences for interpretation of disease trends. PLoS One. 2009;**4**:e4383. DOI: 10.1371/journal.pone.0004383

[97] Miller LH, Baruch DI, Marsh K, Doumbo OK. The pathogenic basis of malaria. Nature. 2009;**415**:673-679. DOI: 10.1038/415673a

[98] Sutherland CJ. The flip-side of cytoadherence immune selection, antigenic variation and the var genes of *Plasmodium falciparum*. Parasitology Today. 1998;**14**:329-332

[99] David PH, Hommel M, Miller LH, Udeinya IJ, Oligino LD. Parasite sequestration in *Plasmodium falciparum* malaria: Spleen and antibody modulation of cytoadherence of infected erythrocytes. Proceedings of the National Academy of Sciences USA. 1983;**80**:5075-5079

[100] Gamain B, Smith JD, Viebig NK, Gysin J, Scherf A. Pregnancy-associated malaria: Parasite binding, natural immunity and vaccine development. International Journal of Parasitology. 2007;**37**:273-283. DOI: 10.1016/j.ijpara.2006.11.011

[101] Fried M, Duffy PE. Adherence of *Plasmodium falciparum* to chondroitin sulfate a in the human placenta. Science. 1996;**272**:1502-1504

[102] Gangnard S, Lewit-Bentley A, Dechavanne S, Srivastava A, Amirat F, Bentley GA, Gamain B. Structure of the DBL3X-DBL4epsilon region of the VAR2CSA placental malaria vaccine candidate: Insight into DBL domain interactions. Science Reports. 2015;**5**:14868. DOI: 10.1038/srep14868

[103] Schofield L. Antidisease vaccines. Chemistry and Immunolology. 2002;**80**:322-342

[104] Lensen AHW, van Druten JA, Bolmer MG, van Gemert GJ, Eling WMC, Sauerwein RW. Measurement by membrane feeding of reduction in *Plasmodium falciparum* transmission induced by endemic sera. Transactions of the Royal Society in Tropical Medicine and Hygiene. 1996;**90**:20-22

[105] Gaudart J, Poudiougou B, Dicko A, Ranque S, Toure O, Sagara I, Diallo M, Diawara S, Ouattara A, Diakite M, Doumbo OK. Space-time clustering of childhood malaria at the household level: A dynamic cohort in a Mali village. BMC Public Health. 2006;**6**:286. DOI: 10.1186/1471-2458-6-286

[106] Pett H, Goncalves BP, Dicko A, Nebie I, Tiono AB, Lanke K, Bradley J, Chen I, Diawara H, Mahamar A, Soumare HM, Traore SF, Baber I, Sirima SB, Sauerwein RW, Brown J, Gosling R, Felger I, Drakeley CJ, Bousema T. Comparison of molecular quantification of *Plasmodium falciparum* gametocytes by Pfs25 qRT-PCR and QT-NASBA in relation to mosquito infectivity. Malaria Journal. 2016;**15**:539. DOI: 10.1186/s12936-016-1584-z

[107] Saul AJ. Efficacy model for mosquito stage transmission blocking vaccines for malaria. Parasitology. 2008;**135**:1497-1506. DOI: 10.1017/S0031182008000280

[108] Okell LC, Paintain LS, Webster J, Hanson K, Lines J. From intervention to impact: Modelling the potential mortality impact achievable by different long-lasting, insecticide-treated net delivery strategies. Malaria Journal. 2012;**11**:327. DOI: 10.1186/1475-2875-11-327

[109] Schneider P, Bousema T, Omar S, Gouagna L, Sawa P, Schallig H, Sauerwein RW. (sub) microscopic *Plasmodium falciparum* gametocytaemia in Kenyan children after treatment with sulphadoxine-pyrimethamine monotherapy or in combination with artesunate. International Journal of Parasitology. 2006;**36**:403-408. DOI: 10.1016/j.ijpara.2006.01.002

[110] Bonnet S, Gouagna LC, Paul RE, Safeukui I, Meunier JY, Boudin C. Estimation of malaria transmission from humans to mosquitoes in two neighbouring villages in South Cameroon: Evaluation and comparison of several indices. Transactions of the Royal Society in Tropical Medicine and Hygiene. 2003;**97**:53-59

[111] Nikolaeva D, Draper SJ, Biswas S. Toward the development of effective transmission-blocking vaccines for malaria. Expert Reviews in Vaccines. 2015;**14**:653-680. DOI: 10.1586/14760584.2015.993383

[112] Greenwood B, Dicko A, Sagara I, Zongo I, Tinto H, Cairns M, Kuepfer I, Milligan P, Ouedraogo JB, Doumbo O, Chandramohan D. Seasonal vaccination against malaria: A potential use for an imperfect malaria vaccine. Malaria Journal. 2017;**16**:182. DOI: 10.1186/s12936-017-1841-9

[113] Sauboin CJ, Van Bellinghen LA, Van de Velde N, Van Vlaenderen I. Potential public health impact of RTS. S malaria candidate vaccine in sub-Saharan Africa: a modelling study. Malaria Journal. 2015;**14**:524. DOI: 10.1186/s12936-015-1046-z

[114] Penny MA, Pemberton-Ross, P, Smith TA: The time-course of protection of the RTS,S vaccine against malaria infections and clinical disease. Malaria Journal. 2015;**14**:437. DOI: 10.1186/s12936-015-0969-8

[115] Renia L, Goh YS. Malaria parasites: The great escape. Frontiers in Microbiology. 2016;**7**: 463. DOI: 10.3389/fimmu.2016.00463

[116] Osier FH, Mackinnon MJ, Crosnier C, Fegan G, Kamuyu G, Wanaguru M, Ogada E, McDade B, Rayner JC, Wright GJ, Marsh K. New antigens for a multicomponent blood-stage malaria vaccine. Science Translational Medicine. 2014;**247**:247ra102. DOI: 10.1126/scitranslmed.3008705

[117] Peng K, Siau A, Franetich JF, Goh YS, Chia WC, Ong ASM, Malleret B, Wu YY, Snounou G, Hermsen CC, Adams JH, Mazier D, Preiser PR, Sauerwein RW, Grüner AC, Rénia L. Breadth of humoral response and sporozoite-neutralizing antibodies are associated with sterile protection induced by controlled human malaria infection. Cellular Microbiology. 2016;**18**:1739-1750. DOI: 10.1111/cmi.12608

[118] Bustamante LY, Powell GT, Lin YC, Macklin MD, Cross N, Kemp A, Cawkill P, Sanderson T, Crosnier C, Muller-Sienerth N, Doumbo OK, Traore B, Crompton PD,

Cicuta P, Tran TM, Wright GJ, Rayner JC. Synergistic malaria vaccine combinations identified by systematic antigen screening. Proceedings of the National Academy of Science USA. 2017;**114**:12045-12050. DOI: 10.1073/pnas.1702944114

[119] Cabrera-Mora M, Fonseca JA, Singh B, Zhao C, Makarova N, Dmitriev I, Curiel DT, Blackwell J, Moreno A. A recombinant chimeric Ad5/3 vector expressing a multistage *Plasmodium* antigen induces protective immunity in mice using heterologous prime-boost immunization regimens. Journal of Immunology. 2016;**197**:2748-2761. DOI: 10.4049/jimmunol.1501926

[120] Camargo TM, de Freitas EO, Gimenez AM, Lima LC, Almeida d, Caramico K, Francoso KS, Bruna-Romero O, Andolina C, Nosten F, Renia L, HCJ E, Nussenzweig RS, Nussenzweig V, Rodrigues MM, Reyes-Sandoval A, Soares IS. Prime-boost vaccination with recombinant protein and adenovirus-vector expressing *Plasmodium vivax* circumsporozoite protein (CSP) partially protects mice against Pb/Pv sporozoite challenge. Science Reports. 2018;**8**:1118. DOI: 10.1038/s41598-017-19063-6

[121] Ewer KJ, Lambe T, Rollier CS, Spencer AJ, Hill AVS, Dorrell L. Viral vectors as vaccine platforms: From immunogenicity to impact. Current Opinion in Immunology. 2016;**41**:43-54. DOI: 10.1016/j.coi.2016.05.014

[122] Garcon N. Di Pasquale a: From discovery to licensure, the adjuvant system story. Human Vaccines and Immunotherapy. 2017;**13**:19-33. DOI: 10.1080/21645515.2016.1225635