

REVIEW

Adjuvants in malaria vaccine development strategies: a review

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Malaria leads in global rates of mortality and morbidity majorly borne by children aged below five years and primigravida women in developing countries. According to WHO, the cases of malaria infections in 2014 declined, despite cases of parasite resistance to available anti-malarial drugs and anopheline mosquitoes resistant to insecticides being reported. Therefore, there is need for an improved malaria control strategy including an effective malaria vaccine which can confer blood stage immunity and prevent development of clinical malaria. This venture has been explored for several decades resulting in discovery of promising antigen candidates but we lack appropriate adjuvants for human use capable of boosting immunogenicity of this antigens. In this review, we highlight limitations of various antigens in conjunction with adjuvants used and provide insight on new strategies of improving vaccine immunogenicity by incorporating immunomodulatory molecules and epitopes.

Keywords: Malaria; adjuvants; immunogenicity; protection; vaccines; clinical trials

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Introduction

Malaria infection is caused by four human infective *Plasmodium* species: *P. falciparum*, *P. ovale*, *P. malariae*, *P. vivax* and one zoonotic simian species *P. knowlesi* ^[1]. Malaria due to *P. falciparum* and *P. vivax* parasites are majorly reported in children aged below five years and primigravida women in developing countries. Globally, in 2014, the World Health Organization (WHO) estimated that 3.2 billion people are at a risk of malaria infection with estimated 438 000 deaths of which 90% occur in SSA ^[2].

The distribution of malaria causing mosquitoes is influenced by climatic factors such as temperature, rainfall and humidity ^[3]. Climatic changes experienced in sub-Saharan Africa (SSA) have altered mosquito vectorial capacity thus influencing prey shifting resulting in transmission of zoonotic diseases ^[4]. Globally, the incidences of malaria are concentrated in tropical and sub-tropical zones of Asia and SSA due to similarity in geographical factors as well as increased levels of poverty and malnutrition thus influencing increased transmission ^[5]. The disease imposes a great economic burden to affected countries through reduced annual per capita which results in reduction of gross national

product (GNP) by more than a half ^[2]. Prevention, control and treatment measures consume the scarce household resources, burden the public health sector affecting child health ^[42]. The global estimate of direct losses occasioned by malaria is about 12 billion dollars annually in SSA ^[6].

A decline in malaria has been reported and this is mainly due to concerted malaria control efforts in the region ^[2]. Malaria eradication and elimination is the main goal, but it is becoming a mirage due to emergence of drug resistant *Plasmodium* parasites and insecticide resistant mosquitoes ^[7]. Armed conflicts, floods, drought, poly-parasitism and co-infections in the region also play a role in hampering control efforts ^[8]. Therefore, an effective malaria vaccine that can confer immunity and prevent development of clinical malaria is urgently needed.

The quest of discovering an effective malaria vaccine dates back to 1925 when first experiments of vaccinating humans with irradiated sporozoites were carried out ^[9]. The available data shows that several malaria antigens have been tested in animal models and humans to determine their safety, immunogenicity and efficacy against various *Plasmodium* strains. The most exciting result is that humans residing in malaria endemic areas acquire natural immunity against clinical disease ^[9]. Passive immunization of immune adults living in malarious areas confers protection against *P. falciparum* in children however, recrudescence of malaria infection has been reported ^[10]. This is in addition to the interesting find that experimental vaccination of humans with attenuated sporozoites confers immunity to patients against subsequent malaria infections ^[11]. Studies utilizing animal models have shown that it is possible to develop an effective malaria vaccine. However these studies have shown efficacy with adjuvants that are not safe in humans. Currently, RTS, S vaccine incorporated with GlaxoSmithKline proprietary adjuvant, AS01 has been licensed for use in some areas of the region despite registering limited efficacy ^[12].

Development of a malaria vaccine is feasible since the *Plasmodium* genome has been completed and shows that there are more than 5200 parasite proteins expressed in its life cycle ^[13]. This provides insight into the development of stage specific malaria vaccines and from this we can have a multi-stage cocktail vaccine. In humans, varied immunological spectacle complicates characterization of malarial antigen immunogenicity ^[14]. Despite these challenges, the use of sub-unit vaccines with adjuvant approaches have been employed in the development of malaria vaccines targeting pre-erythrocytic and erythrocytic stages as well as in transmission blocking or combinations of both ^[15].

Methods

Information sources

The online bibliographic databases, MEDLINE/PubMed, EMBASE, Web of Science, Cochrane Library and Google Scholar® were searched for studies on malaria vaccine development (up to March 2016). Bibliographic lists and references of the selected papers and previous reviews were used as leads for identification of additional studies.

Literature search

Literature search was conducted using predefined medical subject heading (MeSH) terms, Boolean operators (OR, AND), and truncation symbols used with combinations of direct key words: malaria, vaccines, adjuvants, immunogenicity, adverse reactions, blood-stage vaccines, pre-erythrocytic antigens, erythrocytic, transmission-blocking vaccines, multi-strain transcending, licensed vaccines, and all permutations in MeSH terms of all fields.

Study selection

Studies were included in the review if they explicitly reported on malaria vaccine developments in respect to (i) status; (ii) safety of malaria antigens, (iii) immunogenicity of malarial antigens, and (iv) future approaches in malaria vaccine development. All articles selected were managed using Mendeley Desktop reference manager version 1.13.3 (NY, USA). The results of the analysis of the full papers read are described below.

Results

Erythrocytic vaccines

Vaccines that target the *Plasmodia* parasite in blood circulation are designed to mediate immunity by either preventing invasion or sequestration ^[16]. Blood stage vaccine candidates have been explored for decades. These vaccines trigger production of functional antibodies that prevent merozoite invasion or enhance lysis of merozoite-infected erythrocytes ^[1]. Some of the leading vaccine candidates are glutamate-rich protein (GLURP) and merozoite surface proteins (MSPs) ^[17]. However, there are other several vaccine candidates at various phases of field trials in malaria endemic countries of SSA ^[18]. Blood-stage malaria vaccines have received the most attention because this stage of parasite development is symptomatic and the parasites releases a cocktail of antigens that primes the host immune system ^[19]. Blood stage vaccines confer protective immunity and prevent development of clinical disease thus decreasing parasite burden ^[20]. Sometimes a distinction in this type of

vaccine is made between strategies that are anti-parasitic (aiming to inhibit parasite replication) as opposed to those that are anti-toxic (aiming to inhibit the harmful by-products of parasite replication). However, an effective blood-stage vaccine should limit parasite replication and pathogenicity through generation of antibodies aimed at blocking parasite invasion and multiplication in the blood [21]. Available literature, indicates that the naturally acquired antibodies against apical membrane antigen-1 (AMA1) inhibit red blood cells invasion *in vitro* and this has been associated with protection [19]. In cases where alum has been used as an adjuvant to MSP1-C142 [22], AMA1-FVO25-545 [23], AMA1-C1 [24], MSP3154-249, MSP3181-276, SE36 and GLURP85-213 [25] it was noted moderate antibody production, poor cell mediated immunity and did not confer protection during clinical trials. Re-formulations of these antigens with various adjuvants has not improved their immunogenicities [8; 19].

Pre-erythrocytic stage vaccine

Vaccine candidates geared towards preventing hepatocyte infection by sporozoites through production of functional antibodies and induction of potent cytotoxic T-lymphocytes [19]. Recombinant or synthetic expression of short repeated amino acid sequences of circumsporozoite protein (CSP) have been utilized in this kind of vaccines. The *Pf*CS102 antigen with alum has not proved to be immunogenic [14]. However, *Pf*CS102 antigen has good immune responses in combination with AS02, eliciting lymphocyte and cytokine [23]. Similarly, RTS, S vaccine failed when adjuvanted with AS02 but showed some success with AS01 adjuvant [14]. This implies that there have been cases where good malarial antigens have been coupled with inappropriate adjuvants, thus dwarfing their immunogenicities. In addition, genetics of animal model systems have also played a role during pre-clinical trials of malaria vaccines.

Transmission blocking vaccine

These are vaccine candidates designed to block transmission of malaria in the vector's salivary glands interrupting sexual stages of the parasite within the anopheline mosquito [1]. Transmission blocking vaccine candidates elicit antibodies against surface antigens on extracellular parasites that emerge from the infected erythrocytes in the midgut of a mosquito after a blood meal [24]. Antibodies against the gametes prevent fertilization or destroy the gametes or zygotes within 5-10 minutes of entering the mosquito's midgut [1]. Antibodies against ookinetes act 12-24 hours later to prevent them entering the midgut and forming sporozoites, which could eventually infect another host [1]. Leading candidate antigens are

pre-fertilization (*Pfs*230 and *Pfs*48/45), post-fertilization (P25 and P28) and chitinase (petritrophic matrix (PM)) [1]. *P. falciparum* 25 and *P. vivax* 25 antigens have been clinically evaluated in combination with alum. However, this combination has showed low immunogenicity with local reactogenicity [25]. In Phase I of clinical trials, *Pf*25 conjugated with a recombinant *P. aeruginosa* ExoProtein A (EPA) and alum as an adjuvant [19], elicited higher titers of specific antibodies compared to *Pf*25 plus alum alone [26].

Multi-stage malaria vaccine

The use of vaccines made with several *Plasmodia* life cycle antigens is a feasible vaccine that could target multiple development stages of the *Plasmodia* parasite [27]. However, there have been fewer attempts to explore these kinds of vaccines.

Multi-strain transcending vaccines

So far, no malaria vaccine exists that protects humans against the multiplicity of strains that circulate in endemic populations [28]. The fact that individuals living in areas with stable malaria transmission slowly develop naturally acquired immunity, directed to the blood stage of infection, suggests the feasibility of designing effective vaccines [29]. However, the target antigens accounting for this protection are not fully known. *Plasmodia* strains infecting mice and non-human primates confer protection against subsequent heterologous challenge [25] suggesting cross-species immunity mediated by common malarial antigens [30].

It has been suggested that the strain-specific immunity results in slowed development of acquisition of natural immunity [31]. In general, immunization with single malaria vaccine antigens (such as the lead candidates AMA1 and MSP1) exclusively confers protection against challenge with homologous parasites [32]. Antibodies to AMA1 show variable levels of cross-inhibition of parasite growth *in vitro* when tested against heterologous strains of *P. falciparum* [33], suggesting that existing vaccine antigens, of which there are at least 15 blood-stage candidates [30], may not be sufficient when delivered as single vaccines to protect against malaria populations in the field, due to allelic heterogeneity among antigens. Indeed, recent results with a human vaccine containing one allelic form of MSP2 showed that vaccination selected for occurrence of the alternate MSP2 allele in vaccinated individuals [34]. In addition, to be broadly protective, a malaria vaccine will need to circumvent human HLA genetic diversity. Thus, a large panel of T- and B-cell epitopes representing a significant proportion of the antigenic repertoire of the whole parasite should preferably be included in the vaccine [35]. In general, combinations of malarial

antigens acting synergistically provide the greatest protection to challenge infections ^[36].

One strategy to circumvent problems associated with allelic polymorphism in malaria antigens is to focus on conserved antigens/epitopes that can be potentially cross-protective ^[37]. For example, the candidate vaccine antigen MSP4/5 is highly conserved in 14 strains (isolates) of *P. yoelii* (95 to 100% sequence identity), and vaccination with MSP4/5 proteins from two strains (*P. yoelii killicki* and *P. yoelii nigeriensis*) cross-protected mice against challenge with *P. y. yoelii* YM ^[38]. A lesser degree of cross-protection against *P. y. yoelii* was also afforded by vaccination with MSP4/5 from *P. berghei* ANKA (81% sequence identity to *P. yoelii*), but no protection was observed with MSP4/5 from *P. chabaudi adami* DS (55% identity) ^[39]. Such results provide evidence that conserved antigens can elicit good cross-strain protection and even partial cross-species protection. It is notable that several antigens from *P. falciparum* have been shown to cross-protect mice against murine malaria, further demonstrating that cross-species protection is feasible, presumably due to conservation of protective epitopes between species ^[15]; vaccine-induced partial cross-protection among *P. falciparum* strains is also described ^[40].

Adjuvants

In vaccine development, adjuvants play the role of enhancing vaccine immunogenicity, making them stable and safe ^[47]. Safety and appropriateness of an adjuvant for human use is paramount. However, in literature several kinds of adjuvants of unknown safety profiles have been explored during malaria vaccine development ^[41]. The development of the RTS, S with AS02A as an adjuvant has demonstrated the critical importance of appropriate adjuvants and formulations to improve efficacy of sub-unit vaccines. Prior formulations of RTS, S with other adjuvants failed to provide protection in numerous trials ^[47]. However, formulation of RTS, S with a new proprietary adjuvant platform radically changed vaccine efficacy in non-immune, hyper-immune adults, and semi-immune individuals living in malaria-endemic areas of Africa ^[30]. The following adjuvants have been used in malaria vaccine development strategies but they are riddled with several issues on their appropriateness in human use, immunogenicity and stability.

Freund's adjuvant

Prepared from non-metabolizable oils like paraffin and mannide mono-oleate and can also contain killed *Mycobacterium tuberculosis* to improve its immunogenicity ^[32]. The complete Freund's adjuvant (CFA) is designed for continuous antigen release thus inducing persistent immunity

vaccination ^[42]. Complete Freund's adjuvant usage in malaria development has shown some success in inhibiting parasite growth at different stages of the *Plasmodia* life-cycle ^[51]. However, CFA is an inappropriate adjuvant for humans due to its ability to cause adverse reactions by attracting macrophages and other immune cells to the injection site thus causing granulomas and lesions ^[44]. To minimize these side effects, scientists have preferred to use incomplete Freund's adjuvant for boosts compared to CFA that is used during initial injection ^[48].

Alum

These are non-crystalline gels based on aluminium hydroxide, aluminium phosphate or various aluminium salts such as aluminium hydroxyl-sulphate ^[51]. Alum with SPf66 antigen has shown to achieve a 28% reduction in the incidence of new episodes with short-lived antibody responses, low cellular responses and no evidence of protection ^[25]. However, alum has recorded better results with blood-stage vaccines such as: GLURP₈₅₋₂₁₃, AMA1-FVO₂₅₋₂₄₅, AMA1-Cl and MSP1-Cl₄₂ with moderate humoral responses ^[48]. SE36 adjuvanted with alum attains 100% sero-conversion following second vaccination in adults ^[25]. Advantages of alum use is on its safety profile, ability to augment cellular responses, antigen stabilization and relatively simple formulation for large scale production ^[38]. However, alum has inability to induce Th1/CTL type responses required for intracellular pathogens due to lack of depot formation ^[46].

MF59™

In mice studies, PvDBP+MF59™ induces innate and cellular immunity ^[37] while PvBDP+Montanide or AS02A adjuvants, shows enhancement of immunogenicity and blocks red blood cell invasion ^[38]. Moreover, MSP-142 antigen with MF59 barely showed any immune responses in mice ^[25]. Similarly, SERA1 and MF59 fails to confer protection to *Aotus* monkeys following *P. knowlesi* parasites challenge ^[51]. Malaria vaccines adjuvanted with MF59 have not proceeded to clinical trials due to its inability to confer protection following challenge infection ^[38]. However, in comparison with alum, MF59™ has an acceptable safety profile and generates higher antibody titers when coupled with several malarial antigens by inducing greater IgG subclass switching than alum ^[47]. MF59™ stimulates immune cells resulting in lymphocyte, cytokine and chemokine secretion ^[30] but with reduced capacity to induce increased Th1-type immunity ^[39].

MPL

A non-toxic lipopolysaccharide of *Salmonella minnesota* derivative with potential of stimulating Th1 type responses and activates T-cell effector responses^[25]. In malaria clinical trials, strategies of combining of MPL with other adjuvants like alum^[40], QS21 and saponins have been explored and resulted in proprietary adjuvants such as AS04, AS02, AS01, AS01B and AS02A. The use of AS02 with FMP2.1 has shown to influence reduction in parasitaemia levels^[41]. A pivotal Phase III of RTS, S/AS01 has been completed in children/infants (5 to 17 months and 6 to 12 weeks, respectively) showed an efficacy of 55% after a follow up of 12 months^[42]. However, recent results from the latter group (6 to 12 weeks) have evidenced only a 30% efficacy^[43]. Overall, MPL including adjuvant formulations (mainly AS04, AS02, and AS01), are safe and well tolerated despite episodes of local reactogenicity^[30]. This limitation can be reduced by inclusion of natural immuno-stimulants^[43] like chemokines and cytokines^[24].

Immuno-stimulatory oligonucleotides

Adjuvants made of molecular structures present in the pathogens such as the pathogen associated molecular patterns (PAMPS) and can be recognized via the pattern recognition receptors (PRRs) leading to generation of immunological reactions^[44]. Synthetic oligodeoxynucleotides like those containing unmethylated CpG motifs which activate dendritic cells (DCs) to secrete pro-inflammatory and anti-inflammatory cytokines^[45]. Thus, CpG motifs are extremely efficient in inducing Th1/Th1/Treg-type immunity responsible for offering protection against infection, cancer and autoimmune diseases^[46]. Ongoing clinical studies show that tolerance of CpG motifs (TLR ligand 9) varies amongst species thus making evaluation of safety complicated^[47]. A longitudinal study on vaccine with or without CpG motifs has shown that CpG enhances the memory B cell (MBC) kinetics, magnitude and longevity of this response^[26; 48]. The percentage of vaccine specific MBC present at the time of re-immunization predicts vaccine-specific antibody levels 14 days later. At steady state there is a positive correlation between vaccine specific MBC and antibody levels^[32]. In these experiments, evidence was presented that affinity maturation of B cells may fail to occur in the absence of adequate Toll-like receptor (TLR) stimulation^[45]. Therefore, effective malaria vaccines should be coupled with CpG motifs to enhance their immunogenicity. Some patients are relatively refractory to CpG motifs agonists incorporated into malaria subunit vaccines. This raises the possibility that the slow acquisition of MBCs observed may be due to a failure of B cells to undergo affinity maturation during *P. falciparum* infection.^[49]

Imidazoquinolines

These are synthetic compounds that induce dendritic cell maturation, antigen presenting cells induction and chemokine and cytokine secretion^[26, 8]. Imiquimod have been used with P/CS peptide as topical immunomodulators and have been shown to induce production of anti-parasite antibodies in mice^[50]. However, the actual mechanisms has not been explored but it is thought to mimic a microbial antigen thus inducing the expression of pro- and anti-inflammatory cytokines^[30].

Montanides

Montanides are water-in-oil emulsions containing squalene and mannide-monooleate as an emulsifier^[25]. Montanides have been extensively used in malaria, HIV and cancer vaccine trials^[51]. In combination with recombinant malaria proteins such as AMA1 and RAP2, montanides have been shown to influence secretion of high antibody levels in mice, rabbits and monkeys^[32]. In several human malaria studies using recombinant MSP1, MSP2 and RESA in Montanide ISA 720, it promotes strong immune responses^[52]. However, occasional adverse local reactogenicity occurs and this has prevented its use with new malaria vaccine candidates. Also, instability of antigens in ISA 720 formulations has been reported^[19].

Saponins

These are triterpene glycosides isolated from plants^[25]. Quil A is the most widely used in saponin adjuvant extracted from *Quillasa saponaria*^[47]. A purified component of Quil-A, QS-21, has demonstrated low toxicity and maximum adjuvant activity in animals models^[43] when augmented with proteins, glycoproteins and polysaccharide antigens^[53]. It stimulates both humoral and cell mediated Th1-type and CTL responses to subunit antigens^[46]. The use of QS-21 in vaccine candidates has been complicated by its chemical liability of causing adverse reactions at injection sites^[25]. However, it is possible to overcome this limitation by formulating it with other carriers^[37]. Currently, clinical trials with QS-21 alone or in combination with carriers and other immunostimulants (e.g. in AS01 and AS02) for vaccines against infections including malaria are ongoing^[54]. Good immunological profile compared with alum have been recorded with SPf 66 incorporated with QS-21^[15]. However, 2.3% of the individuals vaccinated developed severe vaccine-associated allergies, a significant complication for a prophylactic vaccine^[43].

Immuno-stimulating complexes (ISCOMs)

These complexes are created by combination of an antigen, phospholipids and saponins with micellar cage-like particles

of about 40nm^[32]. The ISCOMs act via hydrophobic interactions with antigen thus endocytosis^[48]. In rabbits, ISCOMs formulation have been reported to induce short-lived humoral responses^[32]. Better humoral responses has been reported with Pf155/RESA, ZZ-M3 and ZZ-M5 when fused with influenza virus derived membrane glycoprotein compared to the use of Freund's adjuvant^[38]. The ISCOMs have not been widely explored during malaria vaccinology due to their instability, difficulty in manufacturing, and associated costs^[48].

Adjuvants for future malaria vaccines

Traditional vaccines were based on whole killed or attenuated microorganisms^[47]. Vaccination with radiation-attenuated sporozoites formed the starting point for investigations into malaria vaccine development^[19]. In humans, complete protection was achieved against *P. falciparum* and *P. vivax* infection by exposing them to mosquito bites whose sporozoites were irradiated and attenuated^[49]. Though highly immunogenic and protective, whole cell vaccines were associated with reversal to pathogenesis, risk of contamination from the infectious material, storage challenges, and batch to batch variation^[42]. Thus, a shift to synthetically designed and genetic-engineered sub-unit vaccines^[50] was plausible. Sub-unit vaccines are safer compared to traditional vaccines and are easier to mass-produce using Good Manufacturing Practice (GMP)^[46]. Unfortunately, these vaccines are known to be poorly immunogenic because they lack PAMPs which are present in attenuated viral or bacterial sub-unit vaccines^[51]. Therefore, effective vaccines require adjuvants that are safe and immunogenic^[42].

Plasmid DNA vaccines and genetically attenuated/transfected parasites are new types of vaccines that are promising and should be explored. Future sub-unit vaccines should allow for protein expression in mammalian cells after introduction of plasmid or recombinant viral vectors encoding the selected protective antigen^[49]. This kind of vaccine has been explored since early 1990s, and has demonstrated induction of protein expression upon direct intramuscular injection of plasmid DNA in myocytes^[51]. Direct *in vitro* and *in vivo* gene transfer of recombinant DNA by a variety of techniques resulted in expression of protein^[14]. Genetic immunization is a novel vaccine strategy that conceptually combines some of the most desirable attributes of standard vaccine approaches^[30].

Plasmid DNA vaccines are being developed as a form of gene therapy that uses a patient's own cellular machinery to make foreign proteins with an aim of mediating induction and maturation of DC as well as deposition of phagocytic

cells at injection site^[50]. Plasmid DNA based vaccines are being considered due to ease of production, low cost, long shelf-life, lack of requirement for a cold chain, and ability to induce both humoral and cellular immune responses^[52]. These stretches of 'naked DNA' contain genes that are easily expressed when the DNA is taken up by muscle and other cells in the body after electroporation^[14].

However, a major shortcoming of plasmid DNA based vaccines in humans and non-human primates has been their sub-optimal immunogenicity when compared with traditional protein-based vaccine approaches^[51]. Therefore, novel strategies are needed to enhance immunogenicity^[30]. The low immunogenicity might be associated with vaccine antigens being rapidly cleared by antigen-specific immune responses or lack of uptake of the DNA into cellular machinery^[14].

The use of genetic as well as immuno-stimulatory adjuvants can be one approach used to improve their immunogenicity^[49]. This is feasible because expression vectors are capable of encoding biologically active molecules such as cytokines, chemokines and co-stimulatory molecules^[19]. Genetic adjuvants can be encoded and co-expressed with malarial antigens or co-expressed on a separate vector and co-injected with the vaccine^[49]. This method provides adjuvant activity at the site of antigen production with long-lasting effect from transfected cells^[44]. The C-C chemokines such as CCL5/RANTES and CCL20/MIP-3 α have proven to work, safe and immunogenic in mice and olive baboons and confers partial cross-species protection^[49]. This is a proof of concept and it has elaborated that chemokine responsiveness and receptor expression play a role in DC recruitment to sites of inflammation and migration to lymphoid organs^[50]. Thus, allowing secretion of chemokine receptors on DCs^[48]. Vaccination with plasmid DNA expressing microbial antigens results in consequent protein expression induction of parasite-specific immunity^[55]. The incorporation of the gene sequence of natural cytokines and chemokines in the plasmids as molecular adjuvants together with the DNA gene sequence of the antigen leads to cytokine expression in the host together with antigen expression^[49]. Cytokines are good chemo-attractants, recruiting APCs resulting in CD4+ and CD8+ cells stimulation and expansion which in turn stimulates a strong humoral and cell mediated immune responses^[55]. Therefore, use of molecular technology should be enhanced in malaria vaccine development.

Concluding remarks

With advancing knowledge in genomics metabolomics and molecular biology, there is hope in developing a strain transcending malaria vaccine. This can be made by

combining the most conserved antigenic epitopes with the highest percentage of identity among the different strains. Genes for these epitopes can be incorporated in plasmid DNA together with natural chemokine genes (as adjuvants) and introduced into the host. Using the host machinery, the *Plasmodia* proteins will be made, and the chemokines will serve as chemo-attractants for recruiting immune cells. With the use of the Olive baboon for pre-clinical trials, reliable data will be obtained to come up with a more efficacious vaccine and correct adjuvant for human use given that baboons share almost 98% protein with humans and hence more likely to mount similar immune and physiological responses.

Conflicting interests

The authors have declared that no conflict of interests exists.

Author contributions

JO and RO designed the study, conducted the literature search, processed the results and drafted the paper. AT, JK, JM and NO guided the design, coordinated the search, did the analysis, drafted and reviewed the paper. All authors read and approved the final paper.

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