

## Malaria Vaccine Development: Recent Advances alongside the Barriers

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### Abstract

Although malaria control efforts have currently reduced much of the morbidity and mortality, yet malaria remains a hard problem in many parts of Africa. Together with the existing malaria control efforts, an effective malaria vaccine is expected to act as an important weapon to fight against malaria burden. Developing an efficacious malaria vaccine is a top priority in the agenda of global health program. However, the multi-stage life cycle, antigenic variation, and vast genetic diversity of malaria parasites made malaria vaccine development difficult for years. This study was conducted with the aim of reviewing the status of malaria vaccine development through different obstacles in the path. Vaccine trials currently upgraded to yield a partially effective and short-lived immunity with the pre-erythrocytic subunit vaccine, RTS,S/AS01, which is likely to be the first vaccine to be licensed for malaria control and elimination in sub-Saharan Africa. A promising result was also observed in the early trials with parenteral administration of the whole sporozoite vaccine in malaria-naïve adults, where a complete protection against malaria was demonstrated. The subunit vaccines may not develop a strong sterile immunity among the malaria endemic community. Therefore, moving forward to the multi-stage vaccine comprising antigens from the pre-erythrocytic, erythrocytic and sexual stages of parasite lifecycle could be the best tool to neutralize the merozoites emerging from hepatocytes and red blood cells, and to break the sexual stage transmission. Furthermore, deep understanding of the potential vaccine targets and how immunity acts is a key roadmap to develop a fully effective vaccine against malaria.

**Keywords:** Malaria control; Plasmodium; Lifecycle; Immunity; Pre-Erythrocytic; Erythrocytic; Transmission blocking; Malaria vaccine

**Abbreviations** AMA: Apical Membrane Antigen; AS<sub>01/02</sub>: Adjuvant System 1/2; CHMI: Controlled Human Malaria Infection; CSP: Circumsporozoite Protein; EBA: Erythrocyte Binding Antigen; ELISA: Enzyme-Linked Immunosorbent Assays; ESV: Erythrocytic Stage Vaccines; GSK: GlaxoSmithKline; ICGEB: International Centre for Genetic Engineering and Biotechnology; KDa: Kilo Dalton; LSAs: Liver Stage Antigens; MSP: Merozoite Surface Protein; MVI: Malaria Vaccine Initiative; MultiMalVax: Multivalent or Multistage Malaria Vaccine; NIAID: National Institute of Allergy and Infectious Diseases; PATH: Programs for Appropriate Technologies in Health; PEV: Pre-Erythrocytic Stage Vaccines; EMP-1: Erythrocyte Membrane Protein-1; PfSPZ: Plasmodium Falciparum Sporozoite Vaccine; RTS,S: The central Repeat region(R) and T Cell Epitopes (T) of CSP, Carried by the Hepatitis B Surface Antigen (HBsAG; S) and Co-expressed within *Saccharomyces Cerevisiae* (S); TBV: Transmission Blocking Vaccines; VMP001: Vivax Malaria Protein 1; WHO: World Health Organization; WRAIR: Walter Reed Army Institute of Research

### Introduction

The mass distribution of insecticide-treated bed nets (ITNs), indoor residual spraying (IRS) and adoption of Artemisinin-based combination therapy (ACT) contributed much for the substantial declines in malaria related deaths globally; however, there are still numerous challenges that should be given due attention [1]. The high genetic diversity of malaria parasites is a great problem which is

resulting in the emergence of new antimalarial drug resistance cases. Despite the emerging resistance, drug discovery program is struggling to avoid the spread of resistance [2]. Similarly, insecticide resistance and immune evasion mechanisms by the parasite are the major threats to malaria control [3]. These all problems call for the development and deployment of new malaria drugs and vaccines to prevent the clinical disease and transmission, and to break off the phases in parasite life cycle.

Most of the malaria research focuses have been on *P. falciparum* because majority of malaria related deaths and morbidities have been caused by this parasite, particularly in Africa [4]. Disease caused by *P. vivax* was considered to be benign for years; severity of the disease was given less concern. However, studies have currently identified complicated malaria and multi-drug resistance cases associated with *P. vivax*. About 25% of patients with complicated malaria in South East Asia were found *P. vivax* mono-infections [5,6]. As a result, the research focus is now being shifted to *P. vivax*; particularly on the disease severity and appearance of drug resistance by the parasite [7].

Supplementary to the existing malaria control efforts, an effective malaria vaccine is an important weapon required to eliminate malaria. Developing an efficacious malaria vaccine is a top priority in the agenda of global health program. Identifying the challenges in the path and updating the status where the malaria vaccine development reached is highly important for the future trials. Therefore, this review was conducted with the aim of updating the status of malaria vaccine development.

## Malaria Vaccine Development

Developing an effective vaccine, which prevents both new Plasmodium infections and clinical disease development, is a major component of the current strategies planned for eradication of malaria worldwide. In most of the malaria endemic regions, two or more *Plasmodium spp* exist together and thus developing the multi-antigen and multi-species-based vaccine is more preferred approach than developing the subunit vaccines. However, the multifaceted malaria parasite life cycle and the high genetic diversity of the parasite are the most important factors acting as the barriers in the path of the development of malaria vaccines [8,9].

Furthermore, each parasitic species shows distinctive biological characteristics, which make the conclusion of lessons learned from one species to the other impossible. For instance, *P. vivax* differentiates into a latent hypnozoite stage in the liver and selectively invades into reticulocytes [10,11]. In the case of *P. falciparum*, all liver schizonts mature and rupture by releasing merozoites that invade the erythrocytes [10,12]. Treatment of symptomatic *P. falciparum* malaria early with anti-blood stage drugs kills both the asexual and sexual parasites; whereas, the *P. vivax* gametocytes can differentiate and be transmitted earlier before the clinical manifestation of malaria. The result of infections varies from person to person and this, too, is one more difficult variation factor in malaria infections [10,11].

In addition, due to the lack of *P. vivax in-vitro* cultures and other limitations, *P. vivax* vaccine development is based on the orthologs of *P. falciparum* antigens [13,14]. The current advance in determining the *P. vivax* genome and proteomic analysis is expected to speed up its vaccine development, by paving the way easier for selection of potential candidate antigens. Development of high throughput techniques for *P. vivax* transfection and platform for population diversity and genetic structure studies are currently ongoing [13].

Developing malaria vaccine includes the selection of potential target antigens or vaccine candidates, developing the product stage and entry into clinical trials [15]. Vaccine development uses target antigens from the three parasite life cycle stages such as the sporozoite and liver stages, asexual blood stages, and the sexual stages. These target antigens are respectively used for developing the three types of malaria vaccines: (1) pre-erythrocytic stage vaccines (PEV), (2) erythrocytic stage vaccines (ESV) and (3) transmission blocking vaccines (TBV) [16].

### Pre-erythrocytic stage vaccines

Pre-erythrocytic stage vaccine (PEV) targets the sporozoites and liver stage parasites and blocks parasitic development to blood-stages [17]. PEVs prevent the entrance of sporozoites into liver cells and thus hinder their maturity into tissue schizonts. The vaccines can also target the early and late liver stage antigens expressed during liver schizogony that possibly induce cell-mediated immunity to inhibit the intracellular parasite growth. This vaccine may be ideal to target the dormant stages (hypnozoites) of *P. vivax* that are responsible for the relapsing nature of the infection [13,17].

### Circumsporozoite protein

Circumsporozoite protein (CSP) is the most abundant protein presented by all Plasmodium species, which is currently the known leading antigen for the development of an anti-sporozoite vaccine [18,19]. CSP entirely blocks infection establishment in the liver [18,19];

it was found capable of preventing clinical malaria as well as transmission. CSP vaccine induces high titer and high avidity antibodies to trap the sporozoites in the skin before their invasion to a blood vessel, and opsonize the antigen for digestion by macrophages or prevent the critical parasitic ligands to the receptors for invasion into hepatocytes. This vaccine could induce long-lasting antibodies or memory B-cells to protect individuals for years, after immunization once in infancy. However, the immunity has disappeared after certain time for unknown reasons. New antigen targets of the CSP protein are upcoming from different immunologic approaches [17,20].

### The RTS, S/AS01 Vaccine

A long and thorough program of malaria vaccine research, after 55 years of trial, has developed a partially effective PEV called the RTS,S/AS01 with 27-46% efficacy. RTS,S/AS01 is the most advanced of malaria vaccines; the trial has been completed recently in the late 2014 [21]. The phase-III randomized trials of RTS,S vaccine recruited 15,459 infants (aged 6 to 12 weeks) and children (aged 5 to 17 months), from 11 study sites with unique malaria transmission across 7 sub-Saharan African countries such as Kenya, United Republic of Tanzania, Malawi, Gabon, Mozambique, Burkina Faso, and Ghana. In 2014, initial phase-III result at 18 months of trial introduction showed the vaccine efficacy of 46% in children and 27% among young infants against the clinical malaria [21].

This vaccine induced both humoral and cellular immunity, with high antibody titers that can block parasitic infection of the liver. The RTS,S/AS01 has been developed by the U.S. military at the Walter Reed Army Institute of Research (WRAIR) through a partnership between GlaxoSmithKline (GSK) Biologicals and the Programs for Appropriate Technologies in Health (PATH) Malaria Vaccine Initiative (MVI). The project was funded by the Bill & Melinda Gates Foundation in the late 1980s [22].

The RTS,S is commercially known as 'Mosquirix'; it is a recombinant protein based vaccine. The vaccine was genetically engineered by using genes from the Repeat region and T cell epitope (RT) of the CSP of the *P. falciparum*, carried by the hepatitis B surface antigen (HBsAG; S) and co-expressed within *Saccharomyces cerevisiae* (S); and hence, the name 'RTS,S' was built. The development of RTS,S has incorporated adjuvant systems, AS01 and AS02, to improve the vaccine efficacy more in trials [23].

The vaccine is so promising although its efficacy is below the malaria vaccine roadmap goal which is to develop a vaccine 50% effective against severe disease and death by 2015 [24] and the next preventing 75% of clinical malaria episodes by 2030 [25]. The clinical trial of RTS,S/AS01 is 5-10 years in front of other candidate malaria vaccines and it is a vaccine for *P. falciparum*, with probably no protection against *P. vivax* [23]. In July 2015, European malaria vaccine agency approved the RTS,S vaccine as the first licensed malaria vaccine for children living in malaria risk areas in sub-Saharan Africa. Further regulatory decisions will be made by the WHO and individual African country governments; then after the product can be rolled out as early as possible in 2017 [9,15].

### The Sanaria® PfSPZ vaccine

Sanaria Inc. has developed for the first time a whole-parasite vaccine, *P. falciparum* sporozoite (PfSPZ), for intravenous vaccination. A phase I clinical trial of the vaccine, which took place from October 2011 to October 2012, demonstrated a completely sterile immunity

(100% protection) against *P. falciparum* in adolescents by controlled human malaria infection (CHMI) in the field with mosquito bites [26]. The developers are Vaccine Research Center (VRC), National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH) and collaborators in the company of Sanaria Inc of Rockville, Bethesda, Maryland, US [26].

In early trial, radiation-attenuated PfSPZ vaccination by intradermal and subcutaneous ways resulted in sterile immunity of more than 90% efficacy in human participants. Consequently, the irradiated sporozoite vaccine has been considered as the gold standard for further vaccination trials. Immunization with irradiated sporozoite has also enabled the identification of the induced protective immune responses and the assortment of numerous vaccine candidates for development. This has encouraged the development of a non-replicating, metabolically active, radiation-attenuated, cryopreserved PfSPZ vaccine by Sanaria Inc [27].

The expected problems in the PfSPZ vaccine trial are the need for multiple doses and administering intravenously which is the uncommon route in vaccination. Researchers are now planning further to evaluate with different doses, to determine whether the vaccine is effective against other strains of Plasmodium parasite and to ascertain how long the protection stays. They also aim to recheck the opportunity that the early subcutaneous or intradermal delivery approaches could give similar protection to the current finding [26].

PfSPZ vaccine clinical trials with IV administration that started in 2013 in Africa, Europe, and the United States are further demonstrating the safety and protective efficacy of the vaccine. Eight trials of two related PfSPZ candidates have been completed in late 2015 or are ongoing further, and 11 more trials have been scheduled to begin in near future in nine countries: Mali, Tanzania, Equatorial Guinea, the U.S, Germany and in others; as could be organized by the regulators in 2017 [26,28,29]. Sanaria is further scheduling to prepare a robot machine for mosquitos' salivary gland dissection, to have much faster and easier vaccine development [30].

### Vivax malaria protein 1

The result of phase 1/2a trial on vivax malaria protein 1 (VMP001/AS01B) in malaria-naïve adults was released on February, 2016 [31]. This vaccine candidate is a novel chimeric protein integrating the amino (N-) and carboxy (C-) repeat regions of CSP and a condensed repeat region of the immunologically different parasitic strains VK210 (type 1) and the VK247 (type 2) [31]. Antibodies induced to the *P. falciparum* CSP have been shown to provide protection against all strains [32,33]. In contrary, the repeat region of *P. vivax* CSP shows sequence heterogeneity indicating that a vaccine based on a single strain may not be fully protective against all strains of *P. vivax* [34], as a result two *P. vivax* strains were used in this candidate.

This vaccine study was conducted at the WRAIR clinical trial center [31], by using a CHMI where the VMP001 was formulated in the GSK Adjuvant System AS01B (500 µL). A total of 30 volunteers in three groups (each group involving 10 individuals) were given three unique types of intramuscular injections of 15 µg, 30 µg, or 60 µg of the VMP001 in AS01B of 500 µL at every immunization. All immunized volunteers participated in a *P. vivax* CHMI 14 days following the third immunization. Six non-vaccinated individuals were used as infectivity controls [31].

The trial showed good tolerance and immunogenicity of the vaccine. All volunteers produced strong humoral and cellular immune

responses to the vaccine. Indeed, the vaccination did not induce complete protection; however, a considerable delay in developing parasitemia was observed in 59% of immunized subjects compared to the controls. An association was seen between the levels of anti-type-1 repeat region specific antibodies and pre-patent period. The study recommended that supplementing the immune responses to the existing domain with other potential immunogens might improve strain-specific vaccine efficacy forward. This trial was the first to evaluate the *P. vivax* CSP vaccine candidate by CHMI. Therefore, accessibility of a *P. vivax* CHMI model will speed up the *P. vivax* vaccine development, allowing better conditions to the field trials [31].

### Liver stage antigens

Liver stage antigens (LSAs) are candidate vaccines targeting infected hepatocytes or antigens in the liver. The weak side of this candidate is that it might induce inflammation in the liver. The LSAs induced cytotoxic and cytokine-secreting CD4+ and CD8+ T cells *in-vitro* that killed parasites growing in the liver [35]. These effector cells can be stimulated by dendritic cells in the lymph nodes draining the mosquito's sporozoite injection sites [36] and then traveling to the liver where they recognize infected cells while presenting sporozoite antigens on the cell surface [37]. *In-vitro* experiment with mice [20] and primates [38] have also demonstrated that effector cells can be induced in the liver by vaccination with live, radiation-attenuated, and genetically weak sporozoites. However, it is mysterious that which of the two T-cell populations, lymph node primed or liver primed, is primarily providing the protection [17].

In the case of *P. falciparum*, LSA-3 is a novel antigen expressed at the pre-erythrocytic stage [39]. A number of studies have demonstrated the potential effect of LSA-3 as a vaccine and serodiagnosis candidate. B- and T-cell epitopes have been characterized in LSA-3 [40], and LSA-3 antigenicity has been demonstrated in several immuno-epidemiological studies conducted in *P. falciparum* malaria-exposed populations [41]. Moreover, an enzyme-linked immune-sorbent assay (ELISA) based on recombinant LSA-3 has been developed as a sero-diagnostic test for *P. falciparum* in Myanmar [42]. There are also preliminary findings, which indicated that the non-replicating viral vectors ChAd63 and MVA encoding for PflSA1 or PflSA2 are capable of inducing complete protection in the presence of CD8+ T-cells in mice. This work has suggested the two promising candidate antigens, PflSA1 or PflSA2, will now undergo further testing in humans [25].

In the case of *P. vivax*, little is known about the liver stage antigens; and the majority of studies conducted after the identification of the pre-erythrocytic stage of *P. vivax* in human liver in 1947 by Garnham [43] focused on the biology of hypnozoites. A recent study for the first time characterized *P. vivax* LSA (PvLSA) using five synthesized peptides located on PvLSA and concluded that the PvLSA candidate may function well during the liver stage of *P. vivax* [24]. Five peptides located inside PvLSA were synthesized, and specific anti-sera to the respective peptides were used to localize PvLSA on *P. vivax* parasites in human liver cells by immunofluorescence. Western blotting and enzyme-linked immunosorbent assays were performed using the five peptides and sera collected from vivax malaria patients and from normal healthy controls. Specific anti-sera produced using the respective peptides were found to react with *P. vivax* parasites in human liver cells. Furthermore, peptides specifically reacted with sera from vivax malaria patients by ELISA [24].

Several LSAs antigen candidates have been selected for development in the form of protein or virally vectored antigens [44]; nevertheless, the trials were found unsatisfactory in inducing protective immunity. Optionally, trials have also proceeded forward by using adenovirus-vectored antigens whose trial have later been discarded in phase I and phase II stages. As a result, researchers are recommending to turn back to a live, genetically attenuated parasites that are administered intravenously [45], because they may revert back to virulence or in fusion with field isolates to yield newly antigenic genotypes [46].

### Erythrocytic stage vaccines

Erythrocytic stage vaccine (ESV) or asexual blood stage vaccines target blood merozoites or infected RBCs, and lowers parasite densities that in turn prevents clinical manifestations and disease severity. ESVs likely may not induce completely sterile immunity due to the parasitic gene alteration and antigenic variation; however, the vaccines were found capable of reducing mortality and morbidity [47]. Some of the ESV trials that have shown the allele-specific protection are merozoite surface protein-2 kilo Dalton (MSP-2 KD), apical membrane antigen-1 (AMA-1) and merozoite surface protein 1 (MSP-1).

The MSP-1<sub>19</sub> and MSP-3 are considered the leading blood-stage vaccine candidates. Antibodies produced against both proteins likely reduced the incidence of *P. falciparum* malaria in some studies. Protective antibodies targeted the C-terminal of MSP-119 and MSP-119 has a highly conserved sequence that elicits cross-reactive antibodies [48]. Recently, International Centre for Genetic Engineering and Biotechnology (ICGEB) in collaboration with the European Vaccine Initiative (EVI) has completed a new Phase Ia trial of MSP-1<sub>19</sub> infusion with the erythrocyte-binding antigen (EBA-175). However, MSP-1<sub>19</sub> is weakly immunogenic because it has no T-cell epitopes and this problem enforced the vaccine research groups to shift the study from 19 KD<sub>a</sub> to the larger 42 KD<sub>a</sub> protein, MSP-1<sub>42</sub>. Further, a fusion protein, MSP-Fu<sub>24</sub>, which comprised of the conserved regions of MSP-3 and MSP-1<sub>19</sub> was developed by ICGEB Malaria Vaccine Group [48]. MSP-3 contains T-cell epitopes that help in boosting antibody responses against MSP-1<sub>19</sub>, which in turn deactivates the growth of parasite by monocyte mediated antibody dependent cellular inhibition (ADCI) [48]. The MSP-3 Phase Ib trial among humans in malaria endemic region induced immunity against clinical malaria. MSP-Fu<sub>24</sub> elicits effective parasite neutralizing antibodies that block RBC invasion and inhibit growth by ADCI, thus making it promising to follow for clinical development [49].

The Ok blood group antigen, Basigin, was found an essential receptor for *P. falciparum* reticulocyte binding protein homolog 5 (PfRh5), a parasitic ligand used for RBC invasion by *P. falciparum* merozoites [12]. This PfRh5 has been found targeting on vaccine inducible & cross reacting antibodies that avoided invasion of merozoites *in-vitro* [50]. Thus, PfRh5 is an essential new viewpoint for erythrocytic vaccine development. Another research roadmap has been towards developing a vaccine against parasitic sequestration of infected RBCs in the maternal placenta. The sequestration has been mediated by the binding of particular *P. falciparum* erythrocytic membrane protein-1 (PfEMP-1) variants to complex polysaccharide receptors in placenta called chondroitin sulfate A (CSA). CSA likely binds with small subset repertoires of the PfEMP-1 variant, probably raising the need for an oligovalent vaccine that may induce antibodies against placental sequestration [51].

In general, blood stage vaccine trials have been disappointing [52] that more than 10 candidate vaccine trials were stopped without

entering into phase 2 trial stage, and only 3 out of the 20 ongoing candidates have reached a phase 2b stage. Majority of merozoite antigens suffer from high levels of allelic variability; thus, two complementary antigen discovery approaches are being used currently: 1) identifying polymorphic antigens that show population level signatures of immune selection and 2) identifying essential, monomorphic or oligomorphic proteins or epitopes that are not naturally immunogenic [52,53]. In both approaches, when the conserved and functionally restricted epitopes are identified and their capacity to develop immunity is improved, there is a possibility that a vaccine flexible to immune selection can be developed to protect against all genotypes of the parasites. Therefore, it is crucial to identify and validate novel blood stage targets that would elicit strain specific inhibitory antibodies.

### Transmission blocking vaccines

Transmission blocking vaccines (TBV) target the sexual stages of the parasite in the blood and in the mosquito. Three types of this vaccine are known to be used *in-vitro*: (i) Pre-fertilization antigens expressed in gametocytes such as Pf230, Pf48/45, and Pf11.1; (ii) Post-fertilization antigens expressed mainly on zygotes or ookinetes such as Pfs25 and Pfs28; and (iii) Late midgut stage antigens such as parasite induced "chitinase" required for the ookinete to infiltrate through the peritrophic membrane. Of the sexual stage vaccine candidates, the major ones are Pfs25, Pfs48/45, and Pfs230. TBV induced antibodies, complements, and cytokines blocked fertilization and parasite development in the mosquito gut, and hence blocked transmission to humans [54,55].

The TBVs are intended to stimulate the production of antibodies in response to the sexual parasites in the blood of TBV-immunized human population; and later these antibodies would be ingested via the blood meal of the mosquito [54]. This vaccine is aimed for use in the control of malaria in the low transmission area, to prevent transmission in the community and to control the parasite mutants that elude from vaccines targeting other parasite stages [54]. TBV could provide 'herd immunity' to lessen transmission; nevertheless, it would not provide individual protection from infection or disease. This may result in difficulty in regulatory processes [54,55], and may cause an influence on the cost-benefit breakdown by national governments and similarly on the suitability of the vaccine to be accepted at the community level. The surface proteins of these life stages are less polymorphic than the asexual stage counterparts and are therefore more satisfying for vaccine development [54].

TBV vaccine might be approved for use based on safety and immunogenicity data without conducting in large-scale randomized trials. However, it is a great challenge when cluster randomized huge community immunization coverage among endemic populations is required. Another difficulty is associated with the complex secondary and tertiary disulfide structures of gamete and gametocyte surface proteins which may hinder expression in their native form and in turn resulting in poor binding of vaccine-induced antibody to the native protein [55]. In addition, a number of antigens expressed during mosquito infection are not expressed in humans, and consequently antibodies induced by vaccination are unlikely to be boosted in a similar way by the successive natural infection [54]. However, the TBV approach is highly appreciated by the global malaria elimination agenda as it is primarily aimed at the current malaria eradication strategy [56].

## Multivalent vaccines

Developing a fully effective malaria vaccine is a great success in the global health research agenda. The multistage or multivalent malaria vaccine (MultiMalVax) is a better option than the subunit malaria vaccines. The subunit vaccines target only the individual stages and thus have a partial immune contribution or immune protection. Because of the high polymorphism in parasite antigen, strain diversity, and immune evasion, malaria vaccine development technology is currently focusing on a MultiMalVax that simultaneously targets several parasite antigens from the three different parasite stages (all pre-erythrocytic, asexual blood stage and sexual stage antigens). In this regard, the ICGEB Malaria Vaccine Group has detected the powerful antigen combinations that elicit inhibitory antibodies against strain-transcending parasite invasion [47]. Thus, ICGEB and other vaccine groups throughout the world are developing new generation MultiMalVax and authorizing their release through different stages [47,57].

Currently, a collaborative MultiMalVax trial led by the Jenner Institute of the Oxford University is ongoing by securing its fund from the European Union's seventh Framework Programme (FP7). The MultiMalVax group started the trial of developing a highly effective multistage malaria vaccine up to proofing the phase II trial in Europe, before starting the further trials in the malaria endemic community [58].

There are promising opportunities recently in vaccine design for all the four *P. falciparum* life cycle stages that promote the trial of multi-component vaccine successfully. These are: i) the foundation of a newly vectored chimpanzee adenovirus vaccination regime that has induced remarkably potent CD8+ T-cell responses and the high titer antibodies against multiple malaria antigens; ii) the development of an enhanced virus like particle (VLP) from parts of RTS,S vaccine candidate, termed R<sub>21</sub>, that lacks excess part of HBsAg in RTS,S; iii) recognition of the blood stage antigen, RH5, by using a vector approach to induce powerful strain-specific antibodies inhibiting the blood stage parasites growth *in vitro* assays; and iv) demonstration of the new nanoparticle vaccine, which have induced antibodies that have blocked 100% of the transmission of the *P. falciparum* in isolates in Africa [58].

## Translational projects and developments

Translational Projects are the selected projects, which have passed their preclinical efficacy trials and are currently committed to their clinical stage of testing. Many new programs are submitted to malaria vaccine initiative (MVI) for preclinical feasibility studies, and only a limited number of projects continue to full translational programs on a clinical trial pathway. Translational stage of development crosses from preclinical studies through Phase 1 to Phase 2 studies. MVI's translational projects of 2015 have been placed below (Table 1).

Project name	Partners	Development stage	Antigens + Adjuvant	Scope
RTS,S/ AS01. (also known as Mosquirix™)	GlaxoSmithKline (GSK); Walter Reed Army Institute of Research; MVI	Phase 3 clinical trials completed in 2014 (1984-2015+)	CSP, HBsAg + AS01E (Adjuvant system)	To elicit humoral immune response to surface exposed sporozoite proteins; Th1 type CMI to free sporozoites in blood [21].
RTS,S-AS01/ ChAd63-TRAP/MVA-TRAP	Oxford University, GSK	Phase 1/2a CHMI clinical trial ended in 2014	CSP, ME-TRAP + AS01	The combined approach will induce both antibody and CMI to CSP and ME-TRAP [59].
Multivalent ChAd63/MVA	Oxford University, Naval Medical Research Center	Phase 1/2a CHMI clinical trial ended in 2013	CSP, ME-TRAP, AMA1+ No adjuvant	To combine partially effective vaccine approaches to increase the levels of protective efficacy in CHMI studies [59,60].
Multivalent pDNA/adenovirus	Naval Medical Research Center, Oxford University	Pre-clinical; TBD	CSP, AMA1, ME-TRAP+ No Adjuvant	In this case the approach uses plasmid DNA to prime and recombinant adenovirus to boost [60].
PvDBP11	International Centre for Genetic Engineering and Biotechnology (ICGEB), the Malaria Vaccine Development Program (MVDP) & and Syngene International Limited	cGMP manufacturing completed by 2014	Region II of the <i>P. vivax</i> Duffy binding protein (PvDBP11) + No adjuvant	Antibodies to PvDBP11 in humans could block red blood cell entry and thus prevent or reduce clinical disease [61,62].
*RTS,S-AS01 fractional dose delayed	GSK, Walter Reed Army Institute of Research (WRAIR)	Phase 1/2a CHMI clinical trial; ends in 2015	CSP + AS01	Repetition using large fractional booster dose to determine the high level of efficacy [32,39].
*Pfs25-VLP	Fraunhofer CMB, Accelovance	Phase 1 clinical trial	Pfs25 + Alhydrogel®	Antibodies block transmission of the parasite from humans to mosquitoes by preventing the parasite from developing in the mosquito [63,64].

**Table 1:** Translational projects currently ongoing by malaria vaccine initiative, 2015. Abbreviations: The Committee for Medicinal Products for Human Use (CHMP); circumsporozoite protein (CSP); hepatitis B surface antigen (HBsAg); chimpanzee adenovirus serotype 63 (ChAd63);

modified vaccinia Ankara (MVA) viral vectors; apical membrane antigen 1 (AMA1); ME-TRAP (multiple malaria epitopes fused to thrombospondin-related adhesion protein); controlled human malaria infection (CHMI); apical membrane antigen 1 (AMA1); International Centre for Genetic Engineering and Biotechnology (ICGEB); Cell Mediated Immunity (CMI); Malaria vaccine initiative (MVI); \*stands for transnational developments based on past results.

## Conclusion

An effective malaria vaccine is an important weapon to fight against malaria burden, together with the current malaria control efforts towards the parasite and vector. Although its efficacy is partial, RTS,S/AS01 vaccine is considered a useful tool against malaria in the children and infants in sub-Saharan Africa where malaria still kills thousands of children every day. The current interest of MVI on developing the multi-stage vaccine comprising pre-erythrocytic, erythrocytic and sexual stages of parasite could be the best tool to overcome parasitic immune evasion mechanism attributed to antigenic polymorphism. The complex life cycle, genetic diversity, and antigenic variation are the major obstacles to the development of vaccines against malaria. Therefore, much better understanding of the interaction between parasite life cycle and the host-pathogen interaction at the molecular level, as well as how immune mechanisms attack parasite strains should be the main road map for the future vaccine development programs.

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## Authors' contribution

DN conceived the study. DN, GT & AA designed the study. DN acquired data. DN & GT participated in data analysis and interpretation. DN drafted the manuscript. AA & GT critically reviewed the manuscript. All authors read and approved the final manuscript.

## Disclosure

The authors report no conflicts of interest in this work.

## References

1. Alonso PL, Tanner M (2013) Public health challenges and prospects for malaria control and elimination. *Nat Med* 19: 150-155.
2. McNamara C, Winzeler EA (2011) Target identification and validation of novel antimalarials. *Future Microbiol* 6: 693-704.
3. Hisaeda H, Yasutomo K, Himeno K (2005) Malaria: immune evasion by parasites. *Int J Biochem Cell Biol* 37: 700-706.
4. Peña-Rosas JP, De-Regil LM, Dowswell T, Viteri FE (2012) Daily oral iron supplementation during pregnancy. *Cochrane Database Syst Rev* 12: CD004736.
5. Tjitra E, Anstey NM, Sugiarto P, Warikar N, Kenangalem E, et al. (2008) Multidrug-resistant *Plasmodium vivax* associated with severe and fatal malaria: a prospective study in Papua, Indonesia. *PLoS Med* 5: e128.
6. Price RN, Douglas NM, Anstey NM (2009) New developments in *Plasmodium vivax* malaria: severe disease and the rise of chloroquine resistance. *Curr Opin Infect Dis* 22: 430-435.
7. Snounou G (2004) Cross-species regulation of *Plasmodium* parasitaemia cross-examined. *Trends Parasitol* 20:262-265.
8. Coulson RMR, Hall N, Ouzounis CA (2004) Comparative Genomics of Transcriptional Control in the Human Malaria Parasite *Plasmodium falciparum*. *Genome Res* 14: 1548-1554.
9. Cullen KA, Mace KE, Arguin PM; Centers for Disease Control and Prevention (CDC) (2016) Malaria Surveillance - United States, 2013. *MMWR Surveill Summ* 65: 1-22.
10. Antinori S, Galimberti L, Milazzo L, Corbellino M (2012) Biology of Human Malaria *Plasmodia* Including *Plasmodium Knowlesi*. *Mediterr J Hematol Infect Dis* 4: 2035-3006.
11. White NJ (2011) Determinants of relapse periodicity in *Plasmodium vivax* malaria. *Malar J* 10: 297.
12. Crosnier C, Bustamante LY, Bartholdson SJ, Bei AK, Theron M, et al. (2011) Basigin is a receptor essential for erythrocyte invasion by *Plasmodium falciparum*. *Nature* 480: 534-537.
13. Arévalo-Herrera M, Chitnis C, Herrera S (2010) Current status of *Plasmodium vivax* vaccine. *Hum Vaccin* 6: 124-132.
14. Carlton JM, Escalante AA, Neafsey D, Volkman SK (2008) Comparative evolutionary genomics of human malaria parasites. *Trends Parasitol* 24: 545-550.
15. Malaria Vaccine Initiative. Rationale for Clinical trials. Steps in malaria vaccine development.
16. MVI PATH. Malaria vaccine approaches. Three types of malaria vaccines.
17. Duffy PE, Sahu T, Akue A, Milman N, Anderson C (2012) Pre-erythrocytic malaria vaccines: identifying the targets. *Expert Rev Vaccines* 11: 1261-1280.
18. The malERA Consultative Group on Vaccines (2011) A research agenda for malaria eradication: vaccines. *PLoS Med* 8:e1000398.
19. Plowe CV, Alonso P, Hoffman SL (2009) The potential role of vaccines in the elimination of *falciparum* malaria and the eventual eradication of malaria. *J Infect Dis* 200: 1646-1649.
20. Matuschewski K, Hafalla JC, Borrmann S, Friesen J (2011) Arrested *Plasmodium* liver stages as experimental anti-malaria vaccines. *Hum Vaccin* 7: 16-21.
21. RTS, S Clinical Trials Partnership (2015) Efficacy and safety of RTS,S/AS01 malaria vaccine with or without a booster dose in infants and children in Africa: final results of a phase 3, individually randomised, controlled trial. *Lancet* 386: 31-45.
22. Heppner DG Jr, Kester KE, Ockenhouse CF, Tornieporth N, Ofori O (2005) Towards an RTS,S-based, multi-stage, multi-antigen vaccine against *falciparum* malaria: progress at the Walter Reed Army Institute of Research. *Vaccine* 23:2243-2250.
23. RTS,S Clinical Trials Partnership, Agnandji ST, Lell B, Fernandes JF, et al. (2012) A phase 3 trial of RTS,S/AS01 malaria vaccine in African infants. *N Engl J Med* 367: 2284-2295.
24. Goo YK, Choi YK, Shin H, Sattabongkot J, So-Young Ji, et al. (2014) First characterization of *Plasmodium vivax* liver stage antigen (PvLSA) using synthetic peptides. *Parasit Vector* 7: 64.
25. Longley RJ, Cottingham MG, Ewer K, Janse CJ, Khan SM, et al. (2015) Comparative assessment of vaccine vectors encoding ten malaria antigens identifies two protective liver-stage candidates. *Sci Rep* 5:11820.
26. Seder RA, Chang LJ, Enama ME, Zephir KL, Sarwar UN, et al. (2008) Protection Against Malaria by Intravenous Immunization with a Nonreplicating Sporozoite Vaccine. *Sci* 2013:1241800.
27. Luke TC, Hoffman SL (2003) Rationale and plans for developing a non-replicating, metabolically active, radiation-attenuated *Plasmodium falciparum* sporozoite vaccine. *J Exp Biol* 206: 3803-8.
28. Sanaria Inc. Malaria Eradication through Eradication.

29. Vaccines R&D (2015) Symposium by Sanaria, Nov 02-05, 2015, Baltimore, USA. PfSPZ-Based Malaria Vaccines from Concept to Licensure.
30. Sanaria Inc to launch crowdfunding campaign for SporoBot™, a mosquito-dissecting robot for accelerating manufacture of Sanaria's malaria vaccine. Rockville, MD –2014.
31. Bennett JW, Yadava A, Tosh D, Sattabongkot J, Komisar J, et al. (2016) Phase 1/2a Trial of *Plasmodium vivax* Malaria Vaccine Candidate VMP001/AS01B in Malaria-Naïve Adults: Safety, Immunogenicity, and Efficacy. *PLoS Negl Trop Dis* 10:e0004423.
32. Kester KE, Cummings JF, Ofori-Anyinam O, Ockenhouse CF, Krzych U (2009) Randomized, double-blind, phase 2a trial of falciparum malaria vaccines RTS,S/AS01B and RTS,S/AS02A in malaria-naïve adults: safety, efficacy, and immunologic associates of protection. *J Infect Dis* 200:337-346.
33. White MT, Bejon P, Olotu A, Griffin JT, Bojang K, et al. (2014) A combined analysis of immunogenicity, antibody kinetics and vaccine efficacy from phase 2 trials of the RTS, S malaria vaccine. *BMC Med* 12:117.
34. Rosenberg R, Wirtz RA, Lanar DE, Sattabongkot J, Hall T, et al. (1989) Circumsporozoite protein heterogeneity in the human malaria parasite *Plasmodium vivax*. *Sci* 245:973-976.
35. Cockburn IA, Zavala F (2007) T cell memory in malaria. *Curr Opin Immunol* 19: 424-429.
36. Chakravarty S, Cockburn IA, Kuk S, Overstreet MG, Sacchi JB, et al. (2007) CD8+ T lymphocytes protective against malaria liver stages are primed in skin-draining lymph nodes. *Nat Med* 13: 1035-1041.
37. Cockburn IA, Tse SW, Radtke AJ, Srinivasan P, Chen YC, et al. (2011) Dendritic cells and hepatocytes use distinct pathways to process protective antigen from *Plasmodium* in vivo. *PLoS Pathog* 7: e1001318.
38. Weiss WR, Jiang CG (2012) Protective CD8+ T lymphocytes in primates immunized with malaria sporozoites. *PLoS One* 7: e31247.
39. Daubersies P, Millet P, Brahimi K, Langermans JA, Ollomo B, et al. (2000) Protection against *Plasmodium falciparum* malaria in chimpanzees by immunization with the conserved pre-erythrocytic liver-stage antigen 3. *Nat Med* 6: 1258-1263.
40. Perlaza BL, Balde AT, Brahimi K, Tall A, Corradin G, et al. (2001) Long synthetic peptides encompassing the *Plasmodium falciparum* LSA3 are the target of human B and T cells and are potent inducers of B helper, T helper and cytolytic T cell responses in mice. *Eur J Immunol* 31: 2200-2209.
41. Toure-Balde A, Perlaza BL, Sauzet JP, Ndiaye M, Aribot G, et al. (2009) Evidence for multiple B- and T-cell epitopes in *Plasmodium falciparum* liver-stage antigen 3. *Infect Immun* 77: 1189-1196.
42. Lee HW, Ryu HS, Kim YJ, Cho SH, Chung GT, et al. (2006) Usefulness of the recombinant liver stage antigen-3 for an early serodiagnosis of *Plasmodium falciparum* infection. *Korean J Parasitol* 44: 49-54.
43. Garnham PC (1987) The liver in malaria with special reference to the exoerythrocytic phase. *Ann Trop Med Parasitol* 81: 531-537.
44. Lindner SE, Miller JL, Kappe SH (2012) Malaria parasite pre-erythrocytic infection: preparation meets opportunity. *Cell Microbiol* 14: 316-324.
45. Epstein JE, Tewari K, Lyke KE, Sim BK, Billingsley PF, et al. (2011) Live attenuated malaria vaccine designed to protect through hepatic CD8 $\alpha^{\circ}$  T cell immunity. *Science* 334: 475-480.
46. Lee SW, Markham PE, Coppo MJ, Legione AR, Markham JF, et al. (2012) Attenuated vaccines can recombine to form virulent field viruses. *Science* 337: 188.
47. Pandey AK, Reddy KS, Sahar T, Gupta S, Singh H, et al. (2013) Identification of a potent combination of key *Plasmodium falciparum* merozoite antigens that elicit strain-transcending parasite-neutralizing antibodies. *Infect Immun* 81: 441-451.
48. Chauhan VS, Yazdani SS, Gaur D (2010) Malaria vaccine development based on merozoite surface proteins of *Plasmodium falciparum*. *Hum Vaccin* 6.
49. Thera MA, Doumbo OK, Coulibaly D, Laurens MB, Ouattara A, et al. (2011) A field trial to assess a blood-stage malaria vaccine. *N Engl J Med* 365: 1004-1013.
50. Douglas AD, Williams AR, Illingworth JJ, Kamuyu G, Biswas S, et al. (2011) The blood-stage malaria antigen PfrH5 is susceptible to vaccine-inducible cross-strain neutralizing antibody. *Nat Commun* 2: 601.
51. Hviid L (2010) The role of *Plasmodium falciparum* variant surface antigens in protective immunity and vaccine development. *Hum Vaccin* 6:84-89.
52. Sheehy SH, Douglas AD, Draper SJ (2013) Challenges of assessing the clinical efficacy of asexual blood-stage *Plasmodium falciparum* malaria vaccines. *Hum Vaccin Immunother* 9: 1831-1840.
53. Weedall GD, Conway DJ (2010) Detecting signatures of balancing selection to identify targets of anti-parasite immunity. *Trends Parasitol* 26: 363-369.
54. Arévalo-Herrera M, Solarte Y, Marin C, Santos M, Castellanos J (2011) Malaria transmission blocking immunity and sexual stage vaccines for interrupting malaria transmission in Latin America. *Mem Inst Oswaldo Cruz* 106: 202-11.
55. Saul A (2007) Mosquito stage, transmission blocking vaccines for malaria. *Curr Opin Infect Dis* 20: 476-481.
56. Schwartz L, Brown GV, Genton B, Moororthy VS (2012) A review of malaria vaccine clinical projects based on the WHO rainbow table. *Malar J* 11: 11.
57. Williams AR, Douglas AD, Miura K, Illingworth JJ, Choudhary P (2012) Enhancing blockade of *Plasmodium falciparum* erythrocyte invasion: Assessing combinations of antibodies against PfrH5 and other merozoite antigens. *PLoS Pathog* 8: e1002991.
58. European Vaccine Initiative. Periodic Report Summary 1- MultiMalVax (A Multi-Stage Malaria Vaccine).
59. Ewer KJ, O'Hara GA, Duncan CJ, Collins KA, Sheehy SH, et al. (2013) Protective CD8+ T-cell immunity to human malaria induced by chimpanzee adenovirus-MVA immunisation. *Nat Commun* 4: 2836.
60. Chuang I, Sedegah M, Ciatelli S, Spring M, Polhemus M (2013) DNA prime/Adenovirus boost malaria vaccine encoding P falciparum CSP and AMA1 induces sterile protection associated with cell-mediated immunity. *PLoS One* 8: e55571.
61. Chitnis CE, Sharma A (2008) Targeting the *Plasmodium vivax* Duffy-binding protein. *Trends Parasitol* 24: 29-34.
62. King CL, Michon P, Shakri AR, Marcotty A, Stanicic D (2008) Naturally acquired Duffy-binding protein-specific binding inhibitory antibodies confer protection from blood-stage. *Plasmodium vivax* infection. *Proc Natl Acad Sci USA* 105: 8363-8368.
63. Kaslow DC, Quakyi IA, Syn C, Raum MG, Keister DB, et al. (1988) A vaccine candidate from the sexual stage of human malaria that contains EGF-like domains. *Nature* 333: 74-76.
64. Jones RM, Chichester JA, Mett V, Jaje J, Tottey S, et al. (2013) A plant-produced Pfs25 VLP malaria vaccine candidate induces persistent transmission blocking antibodies against *Plasmodium falciparum* in immunized mice. *PLoS One* 8: e79538 2013.