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Malaria vaccine clinical trials: what's on the horizon

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Abstract

Significant progress towards a malaria vaccine, specifically for *Plasmodium falciparum*, has been made in the past few years with the completion of numerous clinical trials. Each trial has utilized a unique combination of antigens, delivery platforms, and adjuvants, and the data that has been obtained provides critical information that has poised the research community for the development of next generation malaria vaccines. Despite the progress towards a *P. falciparum* vaccine, *P. vivax* vaccine research requires more momentum and additional investigations to identify novel vaccine candidates. In this review, recently completed and ongoing malaria vaccine clinical trials as well as vaccine candidates that are in the development pipeline are reviewed. Perspectives for future research using post-genomic mining, nonhuman primate models, and systems biology are also discussed.

Introduction

Malaria is the most predominant parasitic infection and continues to have a significant global impact on the health and well-being of hundreds of millions of people annually. In spite of the enormous progress made with reduction in mortality rates in the past 4 years, nearly half of the global population, approximately 3.3 billion people, remains at risk of malaria. About 197 million clinical cases led to 584,000 deaths globally in 2013 [1]. Progress in malaria control interventions, including the use of insecticide-treated nets (ITNs), indoor residual spraying (IRS), chemoprevention and case management, and the growth in funding for malaria control, have resulted in the reduction of transmission intensities and cases. However, recent reports on the development of parasite resistance to front-line malaria drugs such as artemisinins with the threat and spreading of emerging

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multidrug resistant parasites may result in the reversion of this positive trend. Only a combination of malaria prevention tools, diagnostics, chemotherapy and effective vaccines can ensure continued reduction in cases and fatalities and possibly lead to eradication.

Five species of *Plasmodium* infect humans: *P. falciparum*, *P. vivax*, *P. malariae*, *P. ovale* and *P. knowlesi*. *Plasmodium falciparum* and *P. vivax* are the most prevalent and represent a significant global health threat. *Plasmodium falciparum* causes the highest mortality rates worldwide, but *P. vivax* has a wider geographical distribution due to its ability to infect *Anopheles* mosquitoes at lower temperatures. *Plasmodium knowlesi* is a simian malaria parasite that primarily infects humans residing or working in and near forested areas of South-East Asia, where infected macaques reside. This infection is thought to be a zoonosis because human *P. knowlesi* cases have been associated with low density gametocytemia [2]. Low numbers of gametocytes is not advantageous to the parasite because it is less likely to be transmitted to the vector, supporting the thought that human-to-human transmission does not occur. However, more research is needed to determine if these infections are solely zoonotic or if human-to-human transmission can and does occur. Finally, *P. malariae* and *P. ovale* are human malaria parasites that cause mild forms of the disease and typically are observed in coinfections with *P. falciparum* and/or *P. vivax*. Nonetheless, vaccines and better diagnostics for these two species will also be needed for eradication efforts as well.

One of the primary challenges of developing an efficacious malaria vaccine is the parasite's complex life-cycle (Figure 1). Sporozoites, the infectious form of the parasite, are injected into the skin of a susceptible host and then migrate to the liver via the circulation. In the liver, sporozoites invade a host hepatocyte and undergo development and multiplication. Most *Plasmodium* species undergo a single phase of pre-erythrocytic development, but a distinguished feature of *P. vivax* and *P. ovale* infections is the development of a dormant form in the liver known as hypnozoites [3]. The activation of hypnozoites weeks or months after a primary infection is responsible for repeat infections known as relapses. After development in the liver, the parasites are released into the bloodstream where they invade and multiply within host erythrocytes. After multiplication, daughter parasites are released, and this cycle continues until some parasites develop into gametocytes, which are ingested by the vector during a blood meal. Within the *Anopheles* mosquito, the male and female gametocytes fuse to form a zygote that undergoes morphological and developmental changes that result in sporozoites that migrate to the mosquito salivary glands. Each of these steps (i.e. pre-erythrocytic, erythrocytic, and sexual development) are potential targets for vaccines aimed to disrupt the life-cycle, thus, preventing transmission, infection, and/or illness (see below; Figure 1). A multi-stage vaccine targeting two or more of these phases is likely needed to achieve malaria eradication and sterile protection.

Vaccine development efforts have been primarily focused on *P. falciparum*. Changes in malaria epidemiology with increased reports of severe malaria cases due to *P. vivax* infection have been critical factors guiding the malaria research community to establish new research goals for vaccine development. These goals have been published by the World Health Organization (WHO) in the context of the Malaria Vaccine Technology Roadmap [4], which establishes the vision of developing safe and effective vaccines against both *P. falciparum* and *P. vivax*.

Recent clinical trials of *Plasmodium falciparum* vaccines

Pre-erythrocytic vaccines

RTS,S, the most advanced malaria vaccine candidate, has reached phase 3 testing in the clinical trial development pipeline. This vaccine is based on antigenic components of a *P. falciparum* sporozoite surface protein known as the circumsporozoite protein (CSP). The recombinant vaccine antigen includes the repeat region (R) and the complete carboxyl terminal region of the CSP protein, which contains several T cell epitopes (T), fused to the hepatitis B surface antigen (S). To improve the cellular reactivity, the protein is formulated in AS01, an oil-in-water emulsion that incorporates a mixture of MPL (mycobacterial cell wall skeleton) and QS21 (saponin derivative from the soap bark tree *Quillaja saponaria*) [5]. The double-blind, phase 3 trial has been conducted at eleven sites in seven countries across Africa. In total, the trial included 6,537 infants aged 6–12 weeks and 8,923 children aged 5–17 months who received the protein-in-adjuvant RTS,S/AS01 formulation or a control vaccine (meningococcal C vaccine for infants or rabies vaccine for children). Protection against clinical malaria was observed in 46% of children and 27% of infants up to 18 months after vaccination [6]. The vaccine efficacy against severe malaria in children was 34% with no evidence of protection in infants. Interestingly, vaccine efficacy was dependent upon the study site, and higher efficacy was observed in areas with lower incidence of malaria. This finding suggests that vaccine-mediated immunity wanes with time. Therefore, the vaccine's utility in places of high transmission is limited without vector control programs such as ITNs and IRS. Despite these factors, the vaccine has been submitted for regulatory approval by the European Medicine Agency. To improve RTS,S efficacy, alternative vaccination regimens and adjuvants are being explored. Currently, booster doses of the vaccine using a different adjuvant system, namely AS01E (clinical trial registration number (CTRN): NCT02207816), are currently being tested in addition to heterologous prime-boost immunization regimens with recombinant adenovirus (Ad) vectors (CTRN: NCT01366534, Table 1).

Adenovirus vectors have been explored as an alternative to potentiate T cell responses of malaria vaccine candidates. These vectors when used in heterologous prime-boost immunization regimens have the combined advantage of skewing the immune response toward a Th1 profile while also inducing antibody responses and robust CD8 T cells [7]. A heterologous prime-boost immunization using a mixture of DNA plasmid vaccines encoding *P. falciparum* CSP and the *P. falciparum* apical membrane antigen-1 (AMA-1) for priming and a mixture of recombinant Ad5 vectors for boosting immunization showed 27% protective efficacy [8]. However, a subsequent trial including a mixture of the recombinant Ad5 vectors in a single inoculation failed to induce protective efficacy [9]. Differences in protection between these regimens were not linked to the overall magnitude of the cellular immune response but to the relative magnitudes of memory T cell populations, specifically increasing CD8 effector memory relative to the CD8 central memory T cell response [10].

The high frequency of preexisting immunity to Ad5 in humans is a major concern for using recombinant Ad vectors for vaccine development. To address this biological constraint, rare human serotypes or nonhuman (i.e. bovine, canine, ovine, or simian) adenovirus vectors are

employed. A codon-optimized vaccine candidate based on the *P. falciparum* CSP has been expressed in a replication-deficient Ad35, a rare human serotype, [11] and tested in phase 1 clinical trials. A dose-escalation study to assess the safety and immunogenicity of three doses of the vectored vaccine has been reported [12]. The immunization regimens tested induce poor antibody responses with only a four-fold rise from baseline for the highest dose tested (10^{11} virus particles/milliliter). Severe systemic reactogenicity was also reported for volunteers who received higher doses. A dose-escalation trial in semi-immune adults in Burkina Faso resulted in poor antibody responses and moderate induction of IFN- γ and TNF- α secreting CD8 T cells, which are thought to be key correlates of vaccine efficacy [13]. These trials support the need for additional research in effective prime-boost immunization regimens able to induce balanced humoral and cellular immune responses by these vectors.

Multiple epitope-thrombospondin-related adhesion protein (ME-TRAP) is another malaria vaccine candidate that has been genetically incorporated into chimpanzee replication deficient Ad vectors (ChAd63) as well as another common viral vector, the attenuated modified vaccinia virus Ankara (MVA) [14]. This vaccine construct contains a series of B and T cell epitope-encoding sequences from multiple *P. falciparum* liver-stage antigens genetically linked to *trap* sequences. This vaccine has induced unprecedented T cell responses in humans using a heterologous prime-boost immunization regimen [15]. A phase 2a efficacy study using controlled human malaria infections (CHMI) with sporozoites resulted in sterile protection in three out of 14 volunteers (21%) and delays in patency in five more (36%) [16]. A recent comparative efficacy trial using the same vectors to deliver CSP and ME-TRAP demonstrated sterile protection in 13% of the vaccinees immunized with ME-TRAP with a delay in patency in 33%, in contrast with 7% and 20% reported for volunteers immunized with CSP [17]. These findings offer hope that RTS,S can be improved, and clinical trials are underway to test efficacy of the combination of these vaccines in a single regimen as well as the use of the ME-TRAP vectored vaccines in co-administration regimens with RTS,S (CTRN: NCT02252640).

The cell-traversal protein for ookinetes and sporozoites (CeLTOS) is the first vaccine target identified using postgenomic tools, including data mining and differential transcriptome profiling [18]. A codon-optimized protein for expression in *E. coli* based on the *P. falciparum* sequence induced cross-protection using the rodent malaria model *P. berghei* [18]. These results demonstrate promise for such approaches in aiding the identification of potential malaria vaccine candidates in the future. Currently, a protein-in-adjuvant CeLTOS (FMP012)/AS01B formulation is undergoing testing in clinical trials (CTRN: NCT02174978).

The limited efficacy of subunit vaccines reported in clinical trials has provided rationale to revisit the concept of whole organism approaches for pre-erythrocytic malaria vaccine development. Three different approaches have been recently developed: radiation attenuated sporozoites, genetically-attenuated parasites, and sporozoite inoculation under chloroquine prophylaxis (CPS).

Clinical trials with radiation-attenuated sporozoites indicated that intradermal or subcutaneous routes of immunization were not effective [19]. However when administered intravenously, 100% protection (six out of six volunteers) was reported using different doses with the highest being 1.35×10^5 *P. falciparum* irradiated sporozoites delivered five times [19]. Although the route and the immunization regimen are impractical for implementation in endemic areas, several clinical trials in non-immune and semi-immune populations are in progress [20]. Similarly, genetically-attenuated, live *P. falciparum* are under investigation and offer similar prospects in terms of efficacy [21]. A chief obstacle other than dosing regimens is the major facilities and manpower needed to generate substantial amounts of genetically-attenuated parasites for mass-administration, regardless of the attenuation method. Currently, this seems impractical, but through the development of robotics or other engineering methods to dissect mosquitoes, this challenge may be overcome and result in the feasibility of this approach, as long as dosing regimens and administration routes can be optimized.

Dose-response efficacy has been reported using CPS with complete protection in over 80% of volunteers exposed to 30–45 *P. falciparum* infected mosquitoes [22]. High antibody responses against several pre-erythrocytic and erythrocytic antigens were elicited using this vaccination approach [23]. However, antibody responses induced by this immunization strategy were not predictive of protection. Interestingly, this result suggests that immune responses against less immunodominant antigens are important for protection [23] and supports the search for novel antigens that are not necessarily immunodominant. This contrasts with traditional paradigms in vaccinology [24]; however, this approach may be critical for the realization of a malaria vaccine that induces sterile immunity.

Erythrocytic vaccines

The *P. falciparum* erythrocytic-stage merozoite surface protein 1 (MSP-1) and the *P. falciparum* AMA-1 (expressed in merozoites and sporozoites) have also been delivered using ChAd63 and the orthopoxvirus MVA [25]. Protective efficacy against CHMI was tested in volunteers immunized with individual vectored vaccines, a mixture of vectored vaccines or MSP-1 vectored vaccines combined with the pre-erythrocytic vectored ME-TRAP vaccine. Although the vaccines induced robust cellular responses, as expected from such strategies, and moderate IgG responses, no statistically significant efficacy was observed. Unexpectedly, co-administration was associated with immune interference between the two antigens that resulted in reduced antibody and cellular immune responses. A subsequent phase 1 trial, designed to test the combination of priming with the recombinant ChAd63 and boosting with the recombinant MVA and protein-in-adjuvant AMA-1 [26], showed that regimens that include protein-in-adjuvant AMA-1 induced higher antibody responses in comparison to the regimen that only used vectored vaccines. These findings suggest that combined sub-unit and viral-vectored vaccines may be able to induce the appropriate responses needed for protection and provide critical evidence for exploring this strategy in the future.

Recombinant AMA-1 expressed in *E. coli* (FMP2.1) has also been tested in phase 2 clinical trials using a formulation with AS02A [27]. The study population included 400 Malian

children aged 1–6 years at the time of enrollment; enrolled individuals received the protein-in-adjuvant FMP2.1/AS02A formulation or a control rabies vaccine. Although allelic-specific efficacy against malaria caused by the homologous parasite was reported during the first 240 days of follow-up, no efficacy was observed during the follow-up period of 730 days [28]. This lack of efficacy is likely due to the high antigenic diversity of *P. falciparum* AMA-1, which is a common feature of *Plasmodium* proteins that are modulated by host immune selection pressure. Diverse antigens, such as AMA-1, create a moving target for the immune system and are another obstacle in malaria vaccine development. To address the diversity of AMA-1, *P. falciparum* AMA-1 multivalent vaccines that involve more than a single allele have been explored using a combination of recombinant [29] and chimeric proteins that include naturally occurring variants optimized to cover polymorphism [30]. This Diversity Covering (DiCo) vaccine candidate is currently being tested in clinical trials (CTRN: NCT02014727). Such approaches have the advantage of increasing the breadth of antibody responses by redirecting the response toward conserved domains of the protein, which may increase efficacy [31].

A chimeric protein, known as GMZ2, resulting of the fusion of the *P. falciparum* erythrocytic-stage antigens glutamate-rich protein (GLURP) fused to the merozoite surface protein 3 (MSP-3) has been produced in *Lactococcus lactis* and is currently in phase 2b of clinical development. The two components of the vaccine have been previously tested in phase 1 trials as long synthetic peptides, using Montanide ISA720 or Alum as adjuvants. Immunization resulted in the induction of cytophilic antibodies with the ability to inhibit parasite growth *in vitro* [32,33], and functional antibodies, as determined by antibody-dependent cellular inhibition assays [34], can be elicited in malaria-naïve adults and malaria-exposed preschool children after immunization. In contrast to AMA-1, IgG elicited by GMZ2 immunization had similar inhibitory activity against three geographically and genetically diverse *P. falciparum* isolates; thus, this fusion protein may be able to transcend the obstacles associated with the complex diversity of *Plasmodium* antigens.

Transmission blocking vaccines

Transmission-blocking vaccines target pre-fertilization antigens, which are expressed by gametocytes, or post-fertilization antigens, which are expressed by zygotes/oocinets (Figure 1). The vaccines work by preventing infection of the vector and are thought to be critical for malaria eradication. The *P. falciparum* Pfs25 post-fertilization antigen is critical for parasite recognition and attachment to the mosquito midgut, and monoclonal and polyclonal antibodies directed against Pfs25 block the parasite from infecting the mosquito [35]. However, these recombinant vaccine constructs are poorly immunogenic. To improve the immunogenicity of Pfs25, a clinical-grade conjugated vaccine has been recently produced that consists of a recombinant Pfs25 protein expressed in *Pichia pastoris* conjugated to ExoProtein A (EPA), a detoxified form of exotoxin A from *Pseudomonas aeruginosa* produced in *E. coli* [36]. A phase 1a clinical trial is currently underway in Mali to test Pfs25-EPA and a pre-fertilization antigen Pfs230-EPA (CTRN: NCT02334462). Vaccines targeting gametocytes and stages of the parasite within the mosquito are relatively new but may aid in stopping transmission, which is the only way that eradication will ever be realized.

***Plasmodium vivax* preclinical and clinical vaccine trials**

Even though *P. vivax* is widespread and has the added disease burden of a hypnozoite reservoir in the liver, only two *P. vivax* vaccine candidates are currently in advanced stages of clinical trial testing (phase 1 or 2). This contrasts with the twelve *P. falciparum* vaccine trials registered at <http://www.clinicaltrials.gov/> that are currently in a recruiting phase. Nevertheless, some vaccine development progress remains ongoing against vivax malaria. A recombinant protein called *P. vivax* malaria protein 001 (VMP001) that incorporates the complete amino and carboxyl terminal regions of the native CSP protein and a truncated repeat region that contains sequences representing the two allelic forms of CSP has been expressed in *Escherichia coli* and tested in preclinical trials. The chimeric protein is immunogenic in mice and rhesus macaques [37,38] and, protein-in-adjuvant VMP001/Montanide 720 and CpG 10104 formulation induced protective immunity in *Aotus nancymae* monkeys [39]. These animals had 66.7% sterilizing immunity when experimentally challenged with *P. vivax* sporozoites, and the protective efficacy correlated with anti-repeat antibodies [39]. Phase 1 and 2 clinical trials have been completed for this vaccine candidate using a protein-in-adjuvant/AS01B formulation, but the results are not yet available (CTRN: NCT01157897). Another vivax vaccine candidate is based on the interaction of the parasite's Duffy binding protein (DBP) and the Duffy antigen/chemokine receptor (DARC), a critical step in the invasion of red blood cells by *P. vivax* [40]. A viral vectored vaccine based on *P. vivax* DBP using ChAd63 and MVA is currently in clinical trials (CTRN: NCT01816113).

New Candidates on the Horizon

P. falciparum infected erythrocytes are sequestered in the post-capillary vasculature by a mechanism of cytoadherence mediated by adhesive domains expressed by proteins of a family known as *P. falciparum* Erythrocyte Membrane Protein 1 (PfEMP1). Sequestration is directly linked to cerebral malaria, respiratory distress and placental malaria (Reviewed in [41,42]). Studies in areas of intense transmission have indicated that the natural acquisition of PfEMP1 variant-specific antibodies is protective against severe malaria and complications associated with malaria during pregnancy [43,44]. Primigravidas are highly susceptible to clinical complication during pregnancy in contrast with multigravidas that exhibit resistance. The adhesion, responsible for compartmentalization of infected erythrocytes in the placenta, is mediated by the expression of the PfEMP1 variant VAR2CSA [42], and this protein has become the focus of a placental malaria vaccine [45]. A consortium of laboratories established a Pregnancy Malaria Initiative to study specific binding domains of VAR2CSA in-depth using a variety of expression systems [46], and a vaccine candidate based on VAR2CSA has been advancing to clinical testing with support from the European Vaccine Initiative.

The *P. falciparum* reticulocyte-binding protein homolog 5 (PfRh5) has attracted attention as a vaccine candidate over the past few years. This protein is expressed by merozoites, is secreted from the apical organelles during erythrocyte invasion [47] and binds basigin [48]. In contrast with other antigens involved in invasion, PfRh5 exhibits limited genetic diversity. Relevantly, affinity-purified naturally acquired anti-PfRh5 human antibodies

inhibit parasite growth *in vitro* and are associated with reduced risk of malaria [49]. A preclinical trial in *Aotus nancymae* monkeys [50] designed to test the protein's immunogenicity and efficacy using protein-in-adjuvant formulations and viral vectored (ChAd63 and MVA) formulations has been recently reported. However, the best reported outcome was obtained with the protein-in-adjuvant formulation using Freund's adjuvant, which is not suitable for human vaccination. Protective efficacy in this model was correlated with the induction of functional antibodies active in growth inhibition assays. Clinical trials are expected to begin in 2016 [50].

The path forward

This year will be critical for determining if a malaria vaccine candidate, which has received 30 years of research efforts, will be licensed. Although the efficacy and durability of the pre-erythrocytic RTS,S vaccine are far from optimal, the information gathered in the course of several clinical trials has paved the way for the development of a second generation of effective malaria vaccines and has provided continued support for the development of novel subunit vaccines against *Plasmodium*.

Nonetheless, more research and development is needed for materializing an efficacious malaria vaccine using modern technologies. Postgenomic tools have already begun to demonstrate their power to accelerate and enhance malaria vaccine development through the identification and current clinical testing of the subunit vaccine PfCelTOS (see above). The high-throughput technologies that were used to identify this antigen are constantly evolving and continue to offer immense potential for vaccine development. Proteomics, lipidomics, functional genomics, metabolomics and immune profiling give us the unprecedented opportunity to identify novel malaria vaccine candidates, and advance the world towards accomplishing the ambitious goal set-forth by the Malaria Vaccine technology roadmap for developing *P. falciparum* and *P. vivax* vaccines by 2030 with at least 75% efficacy [4]. Furthermore, the use of these tools is also critical to identify vaccine-induced correlates of protection against malaria similarly to what has been done for other vaccines to accelerate vaccine development and clinical testing [51,52].

Systems biology approaches using high-throughput technologies are also needed to identify vaccine candidates for *P. vivax*. Vaccine development for *P. vivax* has lagged behind *P. falciparum* due to biological constraints (e.g. the lack of a long-term, *in vitro* culture system) faced when studying this parasite's biology [53,54]. The identification and characterization of *P. vivax* antigens for all stages of the life-cycle are needed, but the necessity to further our knowledge of hypnozoite biology is at the forefront. Understanding hypnozoite biology is critical to elucidate the underlying mechanism(s) involved in relapse infections and is needed to develop correlates of protection against these forms. These correlates of protection will be essential for assessing the efficacy of *P. vivax* vaccine candidates [55]. Non-human primate models will be critical for obtaining these goals as recently discussed in Joyner et al. [56].

The complexity of the *Plasmodium* life cycle and the mechanisms involved in protection against malaria continue to be major obstacles for vaccine development. In contrast to other

pathogens, such as HIV and *Mycobacterium tuberculosis*, malaria vaccine efficacy can be tested using CHMIs. Although several centers have the technical expertise and logistics in place to run these trials [57–60], anti-malaria chemotherapy is required as soon as parasite are detected in the blood. Thus, CHMIs are unsuitable to characterize the dynamics of the infection, which is needed to understand immune responses resulting in the control and elimination of the parasite and associated disease. Although simian malaria parasites and NHP models have been extensively used in malaria research, their value to characterize the mechanisms involved in malaria pathogenesis and protection has been underestimated.

The impact of genome-based antigen discovery on vaccine development has been recently reviewed by Proietti & Doolan [61]. Such endeavors require major resource investment, but are necessary to capitalize on the extreme benefits of current technologies and move towards systems biology approaches for discovery. The Malaria Host-Pathogen Interaction Center (MaHPIC, <http://www.systemsbiology.emory.edu/>) is an example of a multi-institutional consortium uniquely created to use systems biology approaches to investigate and compare malaria in both humans and non-human primates [62,63]. This consortium is generating unprecedented ‘omics and immune-profiling datasets, for eventual online distribution to the research community, using systems biology tools and several non-human primate - parasite infection combinations as models of malaria. High-throughput technologies are being utilized to capture diverse datasets during the course of infection using simian and human malaria parasite species, and close clinical follow-up involves multiple hematological and parasitological parameters. The integration of the data generated from such infections is supporting systems biology-based antigen and biochemical pathway discovery pipelines, which are essential for the continued search for effective vaccines and new chemotherapeutic agents.

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Highlights

Progress towards a *P. falciparum* malaria vaccine continues with the completion of several trials.

Plasmodium vivax vaccine development needs to be prioritized and pursued.

NHP models and definition of correlates of protection are critical foundations for new vaccines.

Systems biology and post-genomic data mining are essential for the new generation of vaccines.

Figure 1. Malaria Vaccine candidates in clinical trials

Each major stage of the *Plasmodium* life cycle is color-coded, and current vaccines targeting that stage correspond with the colors of the life-cycle. The schematic representation does not include the liver-stage dormant forms known as hypnozoites produced during the pre-erythrocytic stage of *P. vivax* and *P. ovale* infections. Pf, *Plasmodium falciparum* vaccine candidates; Pv, *Plasmodium vivax* vaccine candidates.

Table 1**Malaria vaccine clinical trials pipeline**

Antigen	Vaccine platform	References
<i>P. falciparum</i> CSP (RTS,S)	VLP-in-adjuvant	[5,6]
<i>P. falciparum</i> CSP + <i>P. falciparum</i> AMA-1	DNA/Ad5 prime-boost regimen	[8,9]
<i>P. falciparum</i> CSP	Ad35 vectored	[12,13]
<i>P. falciparum</i> ME-TRAP	ChAd63/MVA prime-boost regimen	[14–17]
<i>P. falciparum</i> ME-TRAP + RTS,S	ChAd63/MVA vectored + VLP-in-adjuvant coadministration	NCT02252640*
<i>P. falciparum</i> CeITOS	Protein-in-adjuvant	NCT02174978*
<i>P. falciparum</i> radiation-attenuated sporozoites	Whole organism	[19,20]
<i>P. falciparum</i> genetically-attenuated sporozoites	Whole organism	[21]
<i>P. falciparum</i> sporozoite inoculation under prophylaxis	Whole organism	[22,23]
<i>P. falciparum</i> MSP-1/ <i>P. falciparum</i> AMA-1	ChAd63/MVA prime-boost regimens	[25,26]
<i>P. falciparum</i> AMA-1 (FMP2.1)	Protein-in-adjuvant	[27,28]
<i>P. falciparum</i> AMA-1 (DiCo)	Protein-in-adjuvant	NCT02014727*
<i>P. falciparum</i> hybrid GLURP+MSP-3 (GMZ2)	Protein-in-adjuvant	[34]
<i>P. falciparum</i> Pfs25-EPA	Conjugated vaccine	NCT02334462*
<i>P. falciparum</i> Pfs230-EPA	Conjugated vaccine	NCT02334462*
<i>P. vivax</i> CSP (VMP001)	Protein-in-adjuvant	NCT01157897*
<i>P. vivax</i> DBP	ChAd63/MVA prime-boost regimen	NCT01816113*

* Clinical Trial Registration Number