SYSTEMATIC REVIEW

Malaria vaccines targeting the pre-erythrocytic stage: a scoping review [version 1; peer review: 2 not approved]

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V1 First published: 07 Jul 2020, 9:680
<https://doi.org/10.12688/f1000research.24320.1>
 Latest published: 07 Jul 2020, 9:680
<https://doi.org/10.12688/f1000research.24320.1>

Abstract

Malaria is a deadly infectious parasitic disease that causes devastating morbidity and mortality globally. Despite being a public health concern, an effective vaccine for prevention of the disease remains elusive. Global efforts are exploring possible ways of developing and improving vaccines to counteract the complex nature in which *Plasmodium falciparum* evades the immune system. A number of vaccines have been developed in the past targeting the various parasitic life cycle stages. Transmission blocker vaccines, such as *PpPf* S25, target the parasite stages in the mosquito vector. However, these herd vaccines only protect the immunized population. Vaccines targeting blood-stage forms, such as the *AMA-1* and *MSP-1* vaccines, are challenged by the complex metabolic pathways of erythrocytes and merozoites. Vaccines targeting the pre-erythrocytic sporozoite stage remain the most promising approach thus far. Here, we systematically review the literature on pre-erythrocytic stage vaccines and on-going work in the field. Furthermore, we highlight gaps in current knowledge and point to potential areas of future work. Articles on pre-erythrocytic malaria vaccines were obtained from Google scholar, PubMed and Cochrane starting from the year 2010. Ten papers were reviewed. A number of vaccines were reviewed highlighting; the vaccine type, clinical phase of trial, population demographics, vaccine immunogenicity, efficacy and safety. The *RTS,S* vaccine is reportedly the most advanced, having been rolled out for phase III clinical trials in a number of malaria-endemic African countries. The pre-erythrocytic vaccines discussed have made milestones in clinical trials. Some of the challenges elicited may be addressed via screening for novel antigens, exploring suitable vaccine

Open Peer Review

Approval Status **XX**

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version 1	XX	XX
07 Jul 2020	view	view

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Any reports and responses or comments on the article can be found at the end of the article.

administration vehicles, as well as using a combined multi-stage vaccine approach.

Keywords

Malaria, Pre-erythrocytic, Plasmodium falciparum, Vaccines, Sporozoites, efficacy

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Competing interests: No competing interests were disclosed.

Grant information: The author(s) declared that no grants were involved in supporting this work.

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How to cite this article: Ogeto T, Ndubi F, Murithi M *et al.* **Malaria vaccines targeting the pre-erythrocytic stage: a scoping review [version 1; peer review: 2 not approved]** F1000Research 2020, 9:680 <https://doi.org/10.12688/f1000research.24320.1>

First published: 07 Jul 2020, 9:680 <https://doi.org/10.12688/f1000research.24320.1>

Introduction

Malaria, a disease commonly found in the tropics and subtropics is caused by parasites of the genus *Plasmodium* whereby *P. falciparum* causes the greatest disease burden. In 2018, it was estimated to have caused 228 million cases globally, out of which 93% (213 million) occurred in Africa¹. In Kenya, malaria is a major public health concern causing 6.7 million cases and 4000 mortalities in children annually^{2,3}. Various community and facility interventional strategies, such as insecticide treated mosquito nets, indoor residual spraying, repellents and chemotherapy have significantly reduced the disease burden over the last decade⁴. However, these strategies are limited in their efficacy; hence elimination of the disease is unlikely without an effective vaccine⁵. Consequently, an effective vaccine conferring high protective efficacy is required to combat the disease.

To date, a number of vaccines have been developed and are at various stages of clinical trials. These vaccines target specific stage(s) of the parasite life cycle, such as pre-erythrocytic (liver-stage), erythrocytic (blood-stage) and gametocytes⁶. The pre-erythrocytic vaccines (*RTS,S*, *PfSPZ*, *TRAP*, *PP*) that target the sporozoites have proved to be the most efficacious of these⁷. Their superiority is founded primarily on the premise that the sporozoites harbour less antigens and are thus easily targeted by an appropriate vaccine. This results in reduced merozoite production thereby rendering the blood stage immune responses superior⁸. The pre-erythrocytic stage is however faced with the challenge of culturing sporozoites *in vitro*, making it difficult to explore novel antigens⁹.

The *RTS,S* vaccine, which has progressed to phase III clinical trials, has reportedly been found to be safe and immunogenic when tested in African children in malaria endemic regions such as Kenya and Tanzania^{10,11}. However, trials of this vaccine done in Africa have revealed low efficacy levels. A study done by Olotu et al (2016), on children aged 5–17 months immunized with *RTS,S/AS01A* vaccine (cases) and rabies vaccine (controls), showed efficacy of 27% against first episode of clinical malaria¹¹. Radiation-attenuated sporozoites (RAS) delivered through the bite of an infected mosquito was 100% protective in humans⁹. An irradiated *P. falciparum* sporozoites (*PfSPZ*) vaccine administered via intravenous injection also resulted in 100% protection in humans¹². Other pre-erythrocytic vaccines that have so far been tested in humans include the thrombospondin related anonymous protein (*TRAP*), multi-epitope thrombospondin related adhesion protein (*ME-TRAP*) and polyprotein (PP) vaccines^{13,14}.

Despite the numerous interventions towards eliminating malaria, the parasite remains elusive. Vaccines remain to be the only hope to combat the disease. Therefore, efforts to understand *P. falciparum* biology and cross-talk mechanisms repressing the host immune response to an active infection are continuously being sought. In this review, we focus on milestones made on the pre-erythrocytic malaria vaccines in the last decade. We further highlight the challenges, immunogenicity and efficacy of these vaccines. Studies included mainly the *RTS,S* vaccine whose clinical trials were carried out in Africa, a malaria endemic region.

Methods

This review was done according to Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA)¹⁵. Articles included were those that had the relevance of the subject of malaria vaccine.

Sources and search strategy

Google Scholar, Cochrane and PubMed were the sources of articles. The search terminologies used were ‘pre-erythrocytic malaria vaccines’ and ‘malaria vaccines’. An advanced search strategy in Google Scholar was done to return articles with all the words ‘pre-erythrocytic’ and ‘malaria vaccines’ in their titles. The exact phrases captured in the article titles were ‘pre-erythrocytic malaria vaccines’ or ‘malaria vaccines’. Article titles without the words ‘review’ or ‘meta-analysis’ or ‘expert opinion’ or ‘models’ dated between 2010–2020 were retrieved. The last search in the databases was done on 24th April 2020.

Eligibility criteria

Clinical trials were the only article type considered; reviews, meta-analysis, expert opinions, *in vitro* studies, mathematical and computer modeling studies were excluded. Articles published in English in the last ten years (2010–2020) were considered since promising milestones have been achieved in the last 10 years.

Selection of articles

To identify the suitability of the article to be included, the titles, abstracts, year of publication and type of study were considered. The papers were screened for eligibility by two reviewers and disagreements were resolved by consensus.

Data extraction

Relevant data was extracted from the included studies based on the type of the vaccine, study design, the clinical trial phase, the mode of vaccine administration, efficacy and safety profile, as well as related adverse events. Other important relevant information, such as the experimental procedures, were also evaluated for each study.

Results

In total, 17 articles were obtained from Google scholar, 7 from Cochrane and 231 from PubMed. After duplicate removal and screening, 10 articles were considered as meeting the criteria (Figure 1).

Table 1 exhibits the characteristics of the 10 articles that were included.

Discussion

The *RTS,S* vaccine is by far the most advanced malaria vaccine in clinical development today. The vaccine confers its protective effect by targeting the circumsporozoite protein (CSP), a specific protein expressed by parasites in the pre-erythrocytic stage of the *Plasmodium falciparum* (Pf) human life cycle⁸. In specific field trials, *RTS,S*-containing vaccines have been administered with either the *AS01* or *AS02* adjuvant systems⁸. By virtue of their intrinsic properties and composition, therefore, vaccines containing the *RTS,S* formulation impart partial pre-erythrocytic

Figure 1. PRISMA flow diagram for screening inclusion and exclusion of studies.

immunity which is distinct from naturally-acquired immunity that mainly targets blood-stage parasites^{8,16}. The latter type of immunity is hypothesized to be mediated mainly by T- and B-cell responses, as well as other possible mechanisms that are not presently well defined. An immuno-epidemiological study conducted by Bejon *et al* in 2011 concluded that vaccination of young children with the *RTS,S/AS01E* vaccine resulted in reduced exposure to blood-stage parasites, therefore reducing anti-merozoite antigen antibody concentrations. The antibodies were, however, not correlates of clinical immunity to malaria⁸.

This is a serious concern associated with use of the *RTS,S* vaccine in the general population.

Several attempts have been made to improve the protective efficacy of the *RTS,S* vaccines. These include, but are not limited to, formulation with more potent adjuvant systems, use of boosted regimens with other CSP-expressing regimens and evaluation of other Pf antigens separately or in combination with *RTS,S*. An example of such a study was conducted using *RTS,S* combined with a recombinant form of thrombospondin

Table 1. Articles (n=10) included in a scoping review of literature on the status of current malaria vaccines.

Vaccine type	Study details	Findings/Results	Reference
Radiation attenuated sporozoite vaccine	<ul style="list-style-type: none"> The vaccine was administered by mosquito bites to true immunized cohorts; Peripheral blood mononuclear cells and plasma samples collected from 10 subjects before and after immunization were used; Negative control group were subjects administered with irradiated non-infected mosquito bites and true immunized subjects were challenged after five, six and seven immunizing doses from bites by non-irradiated mosquitoes carrying infectious sporozoites. 	<ul style="list-style-type: none"> A total of 151 recombinant proteins (RPs) were observed, 131 of which were fully expressed; Of the 151 RPs, 27 were reactive with Western Blots (WBs) when probed with the radiation-attenuated sporozoites (RAS) plasma. The 27 RPs were selected for further profiling and characterization; Protein <i>PF78</i> initially recognized by RAS plasma pool was subsequently not reactive with WBs and was therefore excluded from the initial 27 RPs; Six of the 27 RPs (antigens) failed to induce production of the required threshold of antibodies (Abs) while the remaining 21 were seropositive with sporozoites; 15 of the 21 RPs were positive with 7 day liver stage infection while 13 were positive with blood stage infection and; T-cell immune responses triggered by RAS immunization protected 5 subjects against controlled human malaria infection (CHMI) when assayed by ELISPOT. 	9
RTS,S/ASO: Sub-unit vaccine	<ul style="list-style-type: none"> The vaccine confers pre-erythrocytic immunity and is based on circumsporozoite proteins; A total of 894 children aged between 5–17 months were randomized into 2 groups in the ratio of 1:1 to receive 3 doses of <i>RTS, S/ASO_E</i> or rabies vaccine (CG) at intervals of 1 month. Blood samples from study subjects were collected before vaccination and at 1, 8 and 12 months after the 3rd dose of vaccine; Serum samples were tested on ELISA for presence of human IgG Abs against <i>P. falciparum</i> antigens. 	<ul style="list-style-type: none"> For <i>AMA-1</i>, <i>EBA-175</i> and <i>MSP-1₄₂</i>, Ab levels were highest in youngest subjects and older children; Vaccination with <i>RTS, S/ASO_E</i> was associated with declines in Ab concentration; Infection with clinical malaria episode during 3 months before assaying for Ab levels was associated with 10 fold higher levels of Ab concentration; When adjusted for vaccination group, there was a positive association between increasing Ab concentration and risk of clinical malaria for <i>AMA-1</i>, <i>MSP-1₄₂</i> and <i>EBA-175</i>; Ab concentrations were higher in the higher transmission area and a tenfold increase in Ab concentration was associated with a 13% decrease in the risk of clinical malaria. 	8
PFSPZ: Pf sporozoite attenuated vaccine	<ul style="list-style-type: none"> Open label, to assess efficacy of <i>PFSPZ</i> administered by DVI in 3 (weeks 0, 8 and 16) and 5 (weeks 0, 4, 8, 12 and 20) dose regimens; Subjects were followed up after vaccine injection on days 2, 7 and 14 to assess occurrence of any adverse events; Number of <i>PFSPZ</i> dose, number of doses and intervals between vaccines doses were altered; Assessment of protection was done at week 3 and 24 after administration of the last dose of the vaccine; The assessment was against both homologous and heterologous Pf parasites; Efficiency and tolerability of direct venous inoculation (DVI) of <i>PFSPZ</i> was also assessed. 	<ul style="list-style-type: none"> Short period protection for both heterologous and homologous CHMI was over 80%; Three weeks after completion of the last dose of <i>PFSPZ</i>, 92% of subjects vaccinated with 1.35×10^6 amount in a 5 dose plan and 87% of the subjects vaccinated with 1.35×10^6 amount in a 3 dose plan were protected against homologous CHMI; The trial gave evidence that the vaccine gave short term protection against heterologous CHMI; 24 weeks after completion of the last vaccine dose, 70% and 57% of subjects immunized with the 3-dose and 5-dose of <i>PFSPZ</i>, were protected against homologous CHMI; 10% of subjects administered with 5-dose plan of <i>PFSPZ</i> were protected against heterologous CHMI; <i>PFSPZ</i> vaccine was well tolerated and safe among the immunized subjects: Administration by DVI was most tolerated as compared to other traditional routes. 	12

Vaccine type	Study details	Findings/Results	Reference
RTS,S/ASO₂: Sub-unit vaccine	<ul style="list-style-type: none"> A total of 15,460 children aged 6–12 weeks and 5–17 months were enrolled; Children in each age category were further classified into 3 study groups: the first group received three doses of the vaccine at intervals of 1 month plus a booster dose at 18 months; the 2nd group received 3 doses of the vaccine at intervals of 1 month but were not given a booster dose at 18 months and; the 3rd group received a non-malaria comparator (control) vaccine; The vaccine targets circumsporozoite proteins and is administered with adjuvants (<i>ASO₁</i> or <i>ASO₂</i>); Stage of vaccine: clinical trials; Evaluated efficacy, safety, reactogenicity and immunogenicity of the vaccine across seven countries in Africa; Presented vaccine efficacy against both clinical and severe forms of malaria in the older age category after 12 months of follow-up; Antibody levels were assayed on ELISA; Randomized controlled double blind trial design. 	<ul style="list-style-type: none"> The vaccine's side effect profile was acceptable; It could be administered safely together with other childhood vaccines starting at 6 weeks after birth; The vaccine was immunogenic against both clinical and severe forms of malaria in children who were at least six weeks or older; Incidences of both severe and clinical forms of malaria meeting the primary case definition after 12 months of follow-up among the older age category was 0.44 per/person/year in the <i>RTS, S/ASO₂</i> group while: it was 0.83 per/person/year in the control group (CG); Vaccine efficacy against both forms of malaria was 55.1% at 95% confidence interval (CI); Serious adverse events were observed in 17.6% at (95% CI, 16.7 to 18.6) among subjects in the older age category in the <i>RTS, S/ASO₁</i> group while; 21.6% at (95% CI, 20.1 to 23.1); About 0.9% (95% CI, 0.7 to 1.2) of the older category subjects who received the <i>RTS, S/ASO₁</i> vaccine died while; 0.9% (95% CI, 0.6 to 1.4) in the control group died; Cases of meningitis and other adverse events were reported more in the <i>RTS, S/ASO₂</i> group than in the control group; The geometric mean titer (GMT) of anti-circumsporozoite antibodies were low at enrolment but escalated to 99.9% in the <i>RTS, S/ASO₁</i> group 1 month after administration of the 3rd dose. 	17
RTS, S and TRAP with ASO₂ adjuvants: Sub-unit vaccine	<ul style="list-style-type: none"> Phase 1 trials was conducted in both male and female subjects between the ages 18–50 years, while phase 2 trials were conducted among male and female subjects between ages 18–45 years and who had no history of malaria or injection of an investigative malaria vaccine; In both trials, subjects were to be sero-negative for <i>HBsAg</i> and hepatitis C; In phase I trial, subjects were randomized into three groups as follows: <i>RTS, S/ASO₂</i>, <i>TRAP/ASO₂</i>, and <i>RTS, S/ASO₂/TRAP/ASO₂</i> in the ratio of 1:1:2. The vaccines were administered at 0, 1 and 6 months; Phase II trial was a double blind. Subjects were classified in to 2 cohorts: cohort 1 received sporozoite challenge after 2 doses while cohort 2 was to receive sporozoite challenge after 3 doses. However, because of poor protective exhibited in cohort 1, cohort 2 subjects were not enrolled; Cohort 1 subjects were further randomized to receive: <i>RTS, S + TRAP/ASO₂</i> or <i>TRAP/ASO₂</i> in the ratio of 2:5:1 at 0 and 1 months. The sporozoite challenge was then done at 7 to 30 days after completing the second dose. 	<ul style="list-style-type: none"> The most reported undesirable events were a transient pain at the site of injection, redness and swelling for all groups. No biochemical, hematological and abnormalities associated with urine were reported for during all trials; There were no anti-circumsporozoite antibodies (anti-CS Abs) pre-vaccination. After immunization, geometric mean titers (GMTs) escalated at every time point especially for <i>RTS, S + TRAP/ASO₂</i>; Anti-<i>TRAP</i> GMTs escalated after subsequent doses during the phase 1 trial and decreased 6 months after the 3rd dose; During the 2nd phase, the highest GMT was reported after completion of the 2nd dose for both <i>RTS,S</i> and <i>TRAP/ASO₂</i>; Significant elevations in levels of IFN-γ and IL-5 as well as IL-4 were observed after the 2nd doses for both vaccines. No significant increases in the cytokine levels were reported after the 3rd dose and; Patent parasitemia was detected in all vaccinees upon challenge with sporozoites for all the vaccinees. 	18

Vaccine type	Study details	Findings/Results	Reference
RTS,S/AS0₁ vaccine: a recombinant protein based vaccine	<ul style="list-style-type: none"> • PCR-based next generation sequencing of DNA samples obtained from 4,985 study participants was used to analyze circumsporozoite protein polymorphisms; • Samples representing two protocol-specified end points were sequenced only in participants who received all three vaccinations at months 0, 1, and 2; • The two protocol endpoints included primary clinical malaria and parasite positivity; • Samples extracted from study participants were obtained as dried blood spots on Whatman FTA sample cards and; • The sequencing of circumsporozoite protein C-terminal and SERA-2 amplicons was done on an Illumina MiSeq platform. The sequencing of NANP-NVDP amplicon was done on a PacBio platform since it was longer. 	<ul style="list-style-type: none"> • Most of the samples obtained from participants with primary clinical malaria in the two age bracket, had complex infections; • Distribution of complex malaria infection was skewed towards fewer parasite lineages in those who had received <i>RTS,S/AS0₁</i>; • Among those with high complex malaria, 3D7-matching haplotypes were fewer in those who received <i>RTS,S/AS0₁</i> vaccine compared to those in the control group; • During the 1st year after vaccination, 139 clinical cases of malaria that had a complete full-amplicon 3D7 match and 1951 cases that were mismatched were detected; • Throughout this time in the category of children aged 7–17 months the overall efficacy of the vaccine was 50.3% against clinical malaria that had a complete full-amplicon 3D7 match compared to mismatched malaria where the efficacy was 33.4% and; • In the older age, category the additive vaccine efficacy showed a propensity to reduce as the count of mismatches with 3D7 at these seven amino acid positions reduced. 	16
RTS,S/AS0₁ vaccine: Subunit vaccine	<ul style="list-style-type: none"> • Seven year follow-up of participants who had been randomized in two arms with one arm assigned to get <i>RTS,S/AS0₁</i> vaccine while the other arm received control (rabies) vaccine; • The trial was carried out as part of Phase 2 trial of <i>RTS,S/AS0₁</i> vaccine which was conducted among children that were 5–17 months of age; • Double blinded, randomized controlled trial; • A total of 447 healthy children of ages 5–17 months in 2007 were recruited and followed up for a cumulative duration of seven years; • The participants were assigned to get either <i>RTS,S/AS0₁</i> vaccine or control (rabies vaccine); • Each subject received a total of three doses; at baseline, 1 and 2 months. No other vaccine was administered during the follow up; • In order to check for cases of asymptomatic parasitemia, blood samples were acquired at 8, 12, 15, 25, 38, 65, 78, and 91 months after vaccination; • The primary endpoint was presence of clinical malaria as a result of infection with <i>Pf</i> and presence of <i>Pf</i> in blood at a parasite density of more than 2500/mm³; • Vaccine efficacy was defined as 1 minus the hazard ratio or the incidence-rate ratio multiplied by 100 in the <i>RTS,S/AS0₁</i> group versus the control group. 	<ul style="list-style-type: none"> • The vaccine effectiveness in preventing the index case of clinical malaria was 27.0% (95% CI, 8.5 to 41.8; <i>P</i> = 0.006). Per protocol analysis yielded comparable results; • The approximated effectiveness of the vaccine was 4.4% (95% CI, –17.0 to 21.9; <i>P</i> = 0.66). Comparable results were obtained when per protocol analysis was carried out; • The effectiveness of the trial vaccine appeared to diminish significantly with time. This effect was more pronounced in those participants with high exposure to malaria parasites. The waning effect was also more rapid in the same cohort; • Conduction of intention to treat analysis yielded a vaccine efficacy of 35.9% in the 1st year which declined to 3.6% in the 7th year of follow up; • Overall, the cases of clinical malaria that were avoided due to vaccination with <i>RTS,S/AS0₁</i> vaccine throughout the seven year period was 317 cases per 1000 children who received the vaccine; • Cases averted in the lower exposure group kept on rising throughout the follow up period to a total of 718 cases per 1000 children in the intervention group; • Both arms had similar percentage of children reporting cases of one or more serious adverse effects and so there was no significant difference between the two; • 15 cases of severe malaria were discovered on follow up (5 in the intervention group and 10 in the control group). 	11

Vaccine type	Study details	Findings/Results	Reference
FP9-PP and MVA-PP: A recombinant subunit vaccine.	<ul style="list-style-type: none"> • Malaria naïve healthy adult volunteers of ages 18 to 50 years from Oxford in UK were enrolled into the study; • A total of 34 adults were voluntarily included in the study out of whom 15 were enrolled into the single dose escalation cohorts (1–5) while 19 were included in the prime-boost vaccination cohorts (6 and 7); • The participants in cohort 6 and 7 were challenged with sporozoites of <i>Plasmodium falciparum</i> 2 weeks after the last vaccine administration; • Six control participants without vaccination were voluntarily enrolled and underwent malaria challenge with sporozoites of <i>Plasmodium falciparum</i>; • These participants started visiting the clinic on day 6 after the malaria challenge. Specified symptoms associated with malaria and in addition blood samples for thick blood smear and PCR for <i>Pf</i> were drawn and; • Real-time PCR was carried out at the time of sporozoites challenge and during all the subsequent clinic visits until treatment was initiated. 	<ul style="list-style-type: none"> • The two vaccines were established to be fairly safe even after escalation of the doses; • Immunogenicity elicited by single dose vaccines was low. For the MVA-PP vaccine, higher doses appeared to yield lower responses at the higher dose of 5×10^8 pfu; • In the IFN-γ ELISPOT assay, the immune response against the <i>Pf</i> antigens was less than anticipated in comparison with other studies involving different malaria antigens within the same poxvirus vectors; • All participants enrolled voluntarily and who completed the study ($n=21$) developed clinical malaria evidenced by positive parasitemia on thick smear and had a positive PCR test for <i>Plasmodium</i> malaria; • The vaccines did not retard the onset of parasitemia in those who received the vaccines compared to the control group; • Vaccination appeared to marginally slow down blood stage <i>Pf</i> growth rate. 	14
ChAd63 MVA METRAP: Sub-unit recombinant vaccine	<ul style="list-style-type: none"> • A total of 700 subjects were enrolled. 351 were given 2 doses of ME-TRAP while 349 were given 2 doses of the control vaccine; • Randomized double blind controlled trial; • Study subjects were aged between 5–17 months at the time of vaccine vaccination; • Subjects were randomized in the ratio of 1:1, either to the cohort receiving the ChAd63 ME-TRAP and MVA ME-TRAP or the cohort that received the rabies control vaccine; • The ChAd63 ME-TRAP dose used for purposes of this study was 5×10^{10} vp. The amount of MVA ME-TRAP used for purposes of this trial was 1×10^8 pfu; • A rabies vaccine called <i>Imovax</i> and administered intramuscularly as a single dose was used for control purposes; • Assessing for occurrence of solicited adverse events (AE) was done for 7 days after each round of vaccination while unsolicited AEs were monitored up to a period of 1 month after vaccination for both study and control subjects. Close observation for serious adverse events happened through the study period; • Existence of neutralizing Abs against ChAd63 was not a basis of selection of subjects; • Existence of a blood related, renal or any chronic illness and prior receipt of malaria investigative drugs of vaccine by the target subjects served as a basis of exclusion from study; • Laboratory assays for T-cell responses to ME-TRAP was performed on IFN-γ ELISPOT while; assaying for TRAP specific IgG Abs was performed on ELISA. 	<ul style="list-style-type: none"> • There was notable higher local pain among the vaccine cohorts (15.4%) as compared to the control cohort (0.3%). Other solicited events included: drowsiness, low appetite and fever. The most severe AEs included pneumonia, non-severe malaria, GIT irritation and undernourishment especially to the vaccine cohort than the control cohort; • There were four deaths during the trial, 1 from the ME-TRAP (80th day after vaccination) cohort while 3 from rabies control vaccine cohort. None of the cases was associated with the study vaccines; • IFN-γ Ag specific T-cells escalated among subjects who were vaccinated with ChAd63 MVA ME-TRAP; • At baseline, 2 out of 169 subjects had anti-TRAP IgG Abs; 122 of 169 had anti-TRAP IgG Abs after priming and 165 out of 169 had anti-TRAP after boosting; • A total of 424 subjects had clinical malaria qualifying the primary case definition during the study period. 	13

Vaccine type	Study details	Findings/Results	Reference
RTS,S/AS0₁: A subunit vaccine	<ul style="list-style-type: none"> The design was randomized, double blind phase III trial; Healthy subjects aged between 5–17 months at the time of 1st vaccination were eligible for the study; Vaccination with 3 doses of Hepatitis B vaccines was a requirement for inclusion; A total of 320 subjects were randomized in the ratio of 1:1:1:1 (80 subjects/group) in order to receive 1 of 3 lots or the comparator lot of RTS,S/AS0₁ following a 0, 1 and 2 months schedule; Anti-circumsporozoite Abs (Anti-CS Abs) (IgG) were measured on ELISA before administration of the 1st dose and 1 month after completion of the 3rd dose; Solicited local and general AEs (redness, swelling and pain at injection site and; drowsiness, fever and loss of appetite), unsolicited AEs and serious AEs were trailed for 7 days, 30 days and through the study period respectively. 	<ul style="list-style-type: none"> Anti-CS IgG GMT ratio was between 0.5 to 2 one month after completion of the 3rd dose; The GMT for anti-CS was 271.7EU/ml and 285.8EU/ml for the pilot and commercial lots respectively; Anti-CS prevalence was 3% before vaccination and levels escalated to seropositivity 1 month after completing the last dose of vaccine across all cohorts; Most subjects (≥91.8%) in each cohort had anti-HBs Abs before vaccination; Pain and fever were the most prevalent solicited local and general AEs reported and; 13 unsolicited severe AEs were also reported. 	19

related anonymous protein (*TRAP*) of *Pf* (*PfTRAP*)¹⁸. Measurement of antigen-specific antibody responses, lymphoproliferative responses and IFN production due to the combination vaccine were similar to those produced by each of the single component vaccines. However, both the phase 1 and 2 clinical studies conducted by the investigators indicated inferior protective efficacy with the *RTS,S/TRA*P combination compared to single *RTS,S/AS02* vaccine¹⁸. This was attributed to possible immunological interference, a phenomenon whereby multiple antigens interfere with T-cell priming and restimulation expected with a single antigen. The study was also limited by its significantly small sample size. Future combinations should perform preliminary exclusion of possible immunological interference between component antigens to improve success rates of such 'boosted' regimens.

An important consideration about use of the *RTS,S* vaccines is their duration of protective effect in the general population. Field trials of the vaccine have been conducted on infants and young children, where the vaccine has demonstrated average protection rates of between 18–36% with 3–4 doses of the vaccine administered during a 48-month follow-up period¹¹. These trials were conducted in different African sites with varying rates of malaria transmission. Follow-up studies spanning a period of seven years however produced disappointing results on the long-term protective efficacy of the *RTS,S* malaria vaccine. From the two clinical studies, it was concluded that a three-dose vaccination regimen with *RTS,S/AS01* vaccine offered initial protection against clinical malaria. The result was however offset in subsequent years, especially in areas with higher-than-average exposure to the parasites. Also, anti-merozoite antibody levels in vaccinated individuals were poor correlates of clinical immunity¹¹.

A recent study investigating the relationship between protective efficacy of the *RTS,S/AS01* malaria vaccine and CSP polymorphisms in localized parasite population genotypes indicated that protective efficacy of the vaccine was higher in malaria parasites with matched CSP alleles than those with unmatched alleles¹⁶. This finding is important because CSP polymorphisms and differences in haplotypic regions of the protein exert an effect of vaccine efficacy¹⁶. Significant differences were observed in the cumulative vaccine efficiency rate between different age groups, based on the CSP polymorphisms. Therefore, it may be helpful to incorporate CSP polymorphisms in the local parasite population in future vaccine development efforts. Such an expensive venture would definitely impact on the cost of production, and availability, of these tailor-made malaria vaccines.

On the bright side, a study investigating the immunogenicity and safety of commercially produced *RTS,S/AS01* malaria vaccine on a lot-to-lot basis yielded encouraging results. No significant variations were noted between the *RTS,S/AS01* vaccine lots formulated from a commercial-scale purified antigen bulk batch compared to those formulated from a pilot-scale antigen bulk batch¹⁹. This is an encouraging prospect for future production of multiple batches of the *RTS,S* pre-erythrocytic vaccines

especially in resource-limited settings which are also malaria endemic.

A different approach from the *RTS,S* vaccine adopted in the last decade has led to development of the *Pf* sporozoite (*PfSPZ*) vaccine¹². This vaccine contains radiation-attenuated sporozoites of the parasite, and was assessed for tolerability, safety, immunogenicity and protective efficacy. The *PfSPZ* vaccine was administered by direct venous inoculation of 3–5 doses in non-immune individuals¹². The *PfSPZ* vaccine, given in a three-dose regimen, protected against both 3- and 24-week homologous (similar strain as in vaccine) controlled malaria infections. No significantly alarming side effects were associated with the trialed regimens of this vaccine, and this is an encouraging outcome for future applications of the *PfSPZ* vaccine in mass malaria vaccination campaigns. Upcoming phase III trials of the vaccine will provide further insights on the protective efficacy and safety of the vaccine, especially against heterologous (different strain from vaccine) infections.

Furthermore, a polyprotein (PP) pre-erythrocytic malaria vaccine containing the entire sequence of six separate *Pf* proteins has been investigated. This vaccine was delivered using the combined viral vectors fowl pox virus strain FP9 and modified vaccinia virus Ankara (*MVA*)¹⁴. The design strategy of this 'polyprotein' vaccine was targeted at producing a broad antibody response against a broad range of plasmodial pre-erythrocytic antigens, as opposed to a strong but narrow response. No serious adverse effects were observed with both the *FP9-PP* and *MVA-PP* vaccines, but both failed to induce protection against sporozoite challenge or delay onset of parasitemia in vaccinated individuals¹⁴. The investigators attributed this outcome to possible limits in the size of immunogenic pre-erythrocytic proteins, at least when attached to viral vectors. Future work in the design of PP pre-erythrocytic malaria vaccines should therefore attempt to investigate the theory of size limits to immunogenicity, as well as effects of specific combinations and sizes to efficiency of the viral vectors employed. In addition, a separate phase IIa study utilizing the chimpanzee adenovirus 63 (*ChAd63*) and *MVA* vectors to express a *TRAP*-based vaccine yielded more promising results¹³. Both vaccines were demonstrated to be safe and adequately immunogenic, but induced only moderate T-cell response. This immunogenic response was not sufficient to provide protection against clinical malaria in the follow-up period. Despite these results, viral vectors have a role in malaria vaccine delivery and future work should further investigate novel viral vectors as well as increased applicability in design of pre-erythrocytic vaccines.

Although CSP has stood out over the last few years as an immuno-dominant pre-erythrocytic antigen, several other proteins can be utilized in the design of pre-erythrocytic malaria vaccines. A study of 27 different pre-erythrocytic antigens indicated that 21 out of those, all localized to different parts of the sporozoite, induced detectable antibody responses in animal models⁹. These antigens elicited adequate antibody and T-cell responses and are therefore possible vaccine candidates. In

future development of pre-erythrocytic malaria vaccines, thorough investigation of these additional sporozoite proteins should be undertaken to explore their viability as starting points for vaccine development. It would be interesting to explore these additional proteins as vaccine candidates.

This review had some limitations. We only considered vaccine candidates in clinical trials in the past decade. Therefore, promising vaccine candidates which have not yet been approved for clinical trials were not considered. In addition, only the vaccine candidates affecting the pre-erythrocytic stage were evaluated yet it is possible for a molecule affecting the erythrocytic stage to be an effective malaria vaccine. Articles published in another language apart from English were not included leading to exemption of potentially promising vaccine candidates.

Conclusion

The search for a malaria vaccine targeting the pre-erythrocytic stage of the Pf has led to development of sub-unit vaccines, for example, *RTS,S* vaccine & *ChAd63 MVA METRAP* vaccine and inactivated vaccines like radiation attenuated Pf sporozoite vaccine. However, these vaccines have not been shown to induce sufficient immune responses required to provide adequate protection against malaria. The *RTS,S* vaccine currently undergoing clinical trials remains the most promising. However, its inability to confer long-term protection against clinical malaria and the varied antibody responses associated with the

vaccine remain major drawbacks. The attenuated sporozoite vaccine is also a promising option, and ongoing clinical trials are vital in providing conclusions on the protective effect of this vaccine. Most importantly, ability of the *PfSPZ* vaccine to impart protection against heterologous malaria infections will be vital. Use of viral vectors for malaria vaccines has proven to be a viable mode of delivery. Further work is needed to identify optimal viral vectors for maximum protective efficacy. In addition, the possibility of using combined antigens in a single vaccine and further exploration of other plasmodial antigens as vaccine candidates are both routes that need to be pursued.

Data availability

Underlying data

All data underlying the results are available as part of the article and no additional source data are required.

Reporting guidelines

Harvard Dataverse: PRISMA-ScR checklist for 'Malaria vaccines targeting the pre-erythrocytic stage: a systematic review', <https://doi.org/10.7910/DVN/ZK3ZZH>²⁰.

Acknowledgements

We acknowledge the technical input of Ms. Regina Njeru of the International Livestock Research Institute (ILRI). We are very grateful that she made time to give her valuable contribution.

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<http://www.doi.org/10.7910/DVN/ZK3ZZH>

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<https://doi.org/10.5256/f1000research.26833.r96331>

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Miguel Prudêncio

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The authors set out to write a “scoping review” of pre-erythrocytic malaria vaccines. However, this manuscript does not even remotely cover all relevant aspects of pre-erythrocytic vaccination against malaria. This is hardly surprising, when the authors themselves claim to have reviewed “ten papers” to write this review, when there are hundreds of papers on this subject, and many dozens of these include results on the clinical evaluation of pre-erythrocytic malaria vaccine candidates. It is not even clear how these ten papers were selected, let alone the fact that it is very arguable that they are all among the most relevant in the field. For example, there are no references to genetically attenuated sporozoites (GAS), sporozoites administered under chemoprophylaxis (CPS), Sanaria's PfSPZ-CVAC, a newly described strategy based on genetically modified *P. berghei* parasites expressing *P. falciparum* antigens, or the new subunit vaccine candidate R21 developed by the University of Oxford, all of which have been tested in the clinic. This places this manuscript a very long way from being considered “a scoping review”.

Besides the evident absence of many references to crucial work in this field, the manuscript is also fraught with incorrections or misleading remarks, including in the abstract, such as “transmission blocker (sic) vaccines ... only protect the immunized population” (vaccines usually aim to protect those immunized; what the authors probably meant is that transmission-blocking vaccines are altruistic vaccines that only work at the community level) and “Vaccines targeting blood-stage forms... are challenged by the complex metabolic pathways of erythrocytes and merozoites” (I would argue that the challenges for blood stage vaccines go well beyond the metabolism of erythrocytes and merozoites). Overall, the article falls very short of its stated objective and, in my opinion, does not meet the minimal standards for indexing.

Are the rationale for, and objectives of, the Systematic Review clearly stated?

Partly

Are sufficient details of the methods and analysis provided to allow replication by others?

No

Is the statistical analysis and its interpretation appropriate?

Not applicable

Are the conclusions drawn adequately supported by the results presented in the review?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Pre-erythrocytic stage of *Plasmodium* infection; Malaria vaccines; Antiplasmodial therapeutics

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Reviewer Report 26 October 2020

<https://doi.org/10.5256/f1000research.26833.r71931>

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In this manuscript, Ogeto *et al* attempt to provide a comprehensive review of malaria vaccines targeting pre-erythrocytic stages of *Plasmodium falciparum*. The review falls short of achieving that goal. Although the period from 2010-2020 was considered, only 10 publications were evaluated. The criteria for selection of these articles was not adequately presented. For example 155 articles were deemed not eligible by two reviewers, but the basis for exclusion was not clear. The bulk of the article is Table 1 which simply provides a basic description of each study and a list of findings. In some cases, the information is not adequate to understand the composition of each vaccine formulation and/or to appreciate the primary goal of each study. Overall, the review lacked depth, breadth and a clear theme for an integrated comparison of the papers selected. Considering published reviews covering related topics (i.e. progress on malaria vaccine development, RTS,S, whole sporozoite vaccines), there was little new information or insight provided by the authors. The effort could benefit from a more thorough, critical evaluation of clinical trial results. It is not clear that this summary of pre-erythrocytic malaria vaccine trials will be of substantive value to the field.

In places, the point the authors were trying to convey was not clear. For example, the basis for the following statements is not apparent and/or is confusing:

Abstract: "Vaccines targeting blood-stage forms, such as AMA-1 and MSP-1 vaccines, are challenged by the complex pathways of erythrocytes and merozoites."

Introduction: "Their superiority is founded primarily on the premise that sporozoites harbor less antigens and are thus easily targeted by an appropriate vaccine. This results in reduced merozoite production thereby rendering the blood stage immune responses superior".

Are the rationale for, and objectives of, the Systematic Review clearly stated?

Yes

Are sufficient details of the methods and analysis provided to allow replication by others?

No

Is the statistical analysis and its interpretation appropriate?

Not applicable

Are the conclusions drawn adequately supported by the results presented in the review?

No

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: malaria vaccines

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

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