



# A Systematic Review of Efficacy Outcomes Reported from Clinical Trials Evaluating Vaccine Candidates Targeting *Plasmodium Falciparum*

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## Abstract

**Background:** Malaria remains one of the most destructive communicable diseases worldwide. In the campaign towards global malaria eradication, the development of effective vaccines has become paramount with the emergence of anti-malarial drug resistance to primary treatment and prophylactic regimens. Of particular focus are vaccines specific to *Plasmodium falciparum*. Recently the RTS,S/AS01 vaccine became the first *P. falciparum* vaccine to complete phase III clinical trials. Although these achievements are an important milestone in the quest to eradicate malaria, the efficacy measures reported for RTS,S/AS01 ultimately confirm that RTS,S/AS01 in its current formulation will be insufficient. Myriad other *P. falciparum* vaccine candidates are currently at various stages within the research pipeline. With many already undergoing early evaluation of efficacy in humans.

**Objective:** A systematic review was conducted to evaluate the available efficacy outcomes of *P. falciparum* vaccine candidates currently in clinical trials.

**Methods:** *P. falciparum* vaccine candidates currently in clinical trials were identified using the WHO Rainbow Table. A search of the literature for clinical trial outcomes of efficacy testing was performed using the Pub Med database, the Cochrane Library and the ClinicalTrials.gov website.

**Results:** A total of 21 vaccine candidates, including adjuvant variants, against *P. falciparum* were identified which have been subjected to some degree of efficacy testing in human subjects.

**Conclusion:** Efficacy testing of *P. falciparum* vaccine candidates remains, for the most part, in its early stages. The most promising efficacy outcomes to date have been observed in candidates which specifically target pre-erythrocytic antigens. Further experimentation of vaccines incorporating multiple antigenic targets using combination delivery mechanisms may help leverage full immunogenic potentials; however, translating immunogenicity into efficacy remains challenging.

## Keywords

Malaria vaccine development, Malaria vaccine efficacy, RTS,S/AS01

socioeconomic impact. Over the past fifteen years a renewed global effort targeting *Plasmodium sp.* has led to a 47% reduction in malaria mortality worldwide with a 54% reduction in Africa alone, where 90% of cases are reported [1]. Between 2000 and 2015 it is estimated that the use of insecticide-treated bed nets, artemisinin-based combination therapy and use of sulfadoxine-pyrimethamine prevented between 542-753 million cases of clinical malaria. The most effective treatment and vector control programmes yielding reductions in measures of clinical burden by upwards of 90% [2]. However, replicating equally impressive outcomes is unlikely given that cumulative reduction in incidence attributed to such programs is likely closer to 40% [1,2]. The success of these strategies has reignited interest towards pursuing global eradication and over the last decade many countries have made considerable gains in reducing disease burden while approaching malaria-free status [3]. Unfortunately, it is generally accepted that existing control and treatment strategies will be unable to impede transmission sufficiently to attain malaria-free status in areas where malaria incidence remains high [4]. Subsequently, as part of the revamped global campaign committed to malaria eradication, vaccine development has accelerated rapidly with the 2013 WHO Malaria Vaccine Technology Roadmap setting a long-term strategic goal of developing a vaccine that achieves a minimum efficacy capacity of 75% by 2030 [5]. In 2011, the RTS,S/AS01 vaccine candidate was the first to conclude phase III trials and has since received a 'positive scientific opinion' from the European Medicines Agency (EMA) with policy recommendations pending from the World Health Organization (WHO). Although recommendations will depend on multiple variables, overall vaccine efficacy remains central to conversations regarding cost-benefit of widespread distribution when considering vaccination campaigns.

In addition to RTS,S/AS01, myriad vaccine candidates are currently at various stages of preclinical and clinical trials. The majority of these target *P. falciparum* infection in children less than five years of age, reflecting the significant public health burden of this malarial species on this particularly vulnerable demographic [6]. Although early efficacy measures vary among candidates, outcomes thus far suggest that a future vaccine will be capable of surpassing the efficacy ratings demonstrated by the current formulation of RTS,S and hopefully be able to achieve those goals set out by the 2013 WHO Malaria Vaccine Technology Roadmap. This article will identify the *P. falciparum* vaccine candidates that have reported efficacy results from human trials present the available primary findings and evaluate the changing paradigm of vaccine design targeting *P. falciparum*.

## Introduction

Malaria remains one of the most destructive infectious diseases worldwide imposing an immense burden on many of the most impoverished regions of the world, both in terms of lives lost and

## Anti-malarial immune response and vaccine design

Recovery from a malaria infection, unlike many infectious diseases does not provide sterile immunity. Instead, evidence suggests that naturally acquired immunity is measured by degrees of future protection and immunity against malaria depends on the immune system's capacity to reduce the parasite load in the blood [6]. It is suspected that the immune process is mediated by a variety of antigens expressed during multiple stages of the parasite's development in humans; however, the exact mechanism remains elusive [7]. Further complicating characterization of the immune response is that delineating life cycle stage-specific immune responses has proven difficult in clinical studies [8]. The parasite's life cycle is commonly categorized into 3 stages: pre-erythrocytic, blood stage and sexual stage. Vaccines, in turn, are generally designed with stage-specific antigenic targets and are categorized appropriately.

The pre-erythrocytic stage involves the introduction of malarial sporozoites via the saliva of a female *Anopheles* mosquito during a blood meal. Sporozoites travel to the liver, invade hepatocytes and undergo extensive asexual replication to produce merozoites. It is broadly accepted that pre-erythrocytic immune protection is predominantly a function of cell-mediated immunity (CMI) with only modest involvement of humoral stimulation [9-11]. Pre-erythrocytic vaccine candidates exploit surface antigens expressed by sporozoites. This occurs primarily via two immune mechanisms. First, sporozoite surface antigens are targeted by vaccine induced antibodies, subsequently blocking hepatocyte invasion. Second, sporozoite antigens are presented by MHC-I on infected hepatocytes inducing cell destruction when recognised by CD8+ T cells [12]. Although the pre-erythrocytic stage is metabolically active, it is a symptomatically quiet period of the parasite's life-cycle and can go clinically unrecognized. The role of specific pre-erythrocytic targets in naturally acquired immunity remains incompletely understood, however, by blocking hepatocyte invasion and/or the subsequent release of merozoites, pre-erythrocytic vaccines interrupt parasite development before blood-stage infection and thus before onset of clinical symptoms [13].

While the pre-erythrocytic response targets sporozoites and liver-stage antigens, the blood-stage response targets merozoites and intra-erythrocytic parasites. During the blood-stage merozoites are released into the blood stream from hepatocytes. This leads to repeated cycles of: i) erythrocyte invasion, ii) intracellular maturation and asexual multiplication, iii) erythrocytic lysis, iv) release of increasing numbers of merozoites, and v) re-invasion of erythrocytes. Blood-stage infection induces multiple immune responses, the most prominent of which is a humoral response characterised by the production of antibodies capable of blocking erythrocytic invasion by merozoites [7,14]. Cell-mediated and antibody-dependent responses have also been identified although the mechanism of the former remains poorly characterised considering erythrocytes do not express MHC classes I or II [15-18]. Blood-stage vaccine candidates have thus more commonly used recombinant protein-in-adjuvant design constructs using well-characterised merozoite surface antigens to stimulate a predominately humoral response [19-22].

During the blood-stage cycle, some merozoites escape the cycle of asexual reproduction and transform into gametocytes. These can be re-ingested by a feeding mosquito and subsequently enter sexual reproduction. This leads to a new generation of sporozoites that migrate to the mosquito's salivary glands and subsequently injected into a new host during a blood meal. Vaccines targeting the sexual life-stage are commonly referred to as transmission blocking vaccines (TBV) as they interrupt parasitic reproduction within a mosquito host. The Pf25 vaccine, currently in phase 1 trials and the most advanced TBV, induces antibody production against antigens that only present during sexual reproduction within a mosquito host. These antibodies are ingested during a blood meal and will block target antigens once expressed, thereby impeding progression from gametocyte to sporozoite [23].

## Pre-erythrocytic stage vaccines in efficacy testing

The *Plasmodium falciparum* (Pf) sporozoite (SPZ) vaccine candidate is an attenuated whole sporozoite incapable of replication. Reflecting the natural progression of the malarial sporozoite, the PfSPZ vaccine acts by traveling to the liver, invading hepatocytes and activating a potent CD8+ T cell response. However, it is unable to mature beyond the liver-stage. Previous research into the use of attenuated sporozoites has yielded unprecedented short-term outcomes. One study reported that all volunteers who received greater than 1000 immunising bites from irradiated mosquito's infected with a homologous *P. falciparum* strain demonstrated full protection for at least 9 weeks following challenge with mosquitoes infected with *P. falciparum* [24]. Whole sporozoite vaccines are the only vaccine candidate to date to demonstrate greater than 90% efficacy against malaria. However, until recently with the introduction of new techniques capable of manufacturing and cryopreserving PfSPZ, vaccine development using whole sporozoites appeared impractical due to the requirement for inoculation via mosquito bite and the immense number of attenuated sporozoites required to induce the desired protective immunity [25,26].

The RTS,S/AS01 monovalent subunit vaccine has completed phase III trials in children aged 5 to 17 months and infants aged 6 to 12 weeks across 11 sites in Africa. RTS,S is a composite of the carboxy-terminal of the *Plasmodium* sporozoite circumsporozoite (CS) surface protein bound to the hepatitis B surface antigen (HBsAg), while AS01 is a common adjuvant system designed to boost the vaccinees immunogenicity [27,28]. The CS protein functions as a surface adhesion molecule with hepatocyte specificity and has been associated with facilitating cellular invasion. It is believed to play a central role in infectivity in both the salivary gland of mosquitoes and in the human liver [29]. Current evidence suggests that the immune response induced by RTS,S/AS01 is primarily humoral, stimulating induction of anti-CS IgG antibodies [30]. It is suggested that an additive, albeit minor, role of CS-specific CD4+ T cells is involved [31,32] with an even weaker or near absent CS-specific CD8+ T cell response [33].

The heterologous prime-boost approach employed by the Chimpanzee Adenovirus 63 - Modified vaccinia Virus Ankara (ChAd63-MVA) plasmid vector uses a gene-vector delivery mechanism and is designed to potentiate a more robust cell-mediated response as protein-in-adjuvant design models have failed, for the most part, to do so. The ChAd63-MVA plasmid vector vaccine employs a prime-boost mechanism which involves administration of a primary target antigen via one vector system (ChAd63), followed a few weeks to months later by a second vector (MVA) encoded with the same target antigens. This secondary MVA "booster" is specifically designed to strengthen the cell-mediated response [34]. To date, two pre-erythrocytic vaccine candidates using the ChAd63-MVA vaccine model have undergone efficacy testing [35]. ChAd63-MVA CS and ChAd63-MVA ME-TRAP. ChAd63-MVA CS encodes circumsporozoite protein while ChAd-MVA ME-TRAP encodes the multiple-epitope string\* thrombospondin-related adhesion protein (ME-TRAP). ME-TRAP is a surface protein expressed by sporozoites and is believed to contribute significantly to hepatocyte invasion [36,37]. ChAd63 ME-TRAP has also been evaluated for efficacy testing as a standalone single dose vaccine.

The Multi-stage DNA Vaccine Operation, 5 genes (MuStDO5) vaccine is a multivalent DNA vaccine composed of a mixture of five plasmids. These plasmids each encode a different erythrocytic surface antigen gene, including: PfCSP, PfSSP2/TRAP, Pf exported protein-1 (PfEXP1), Pf liver stage antigen-1 (PfLSA1) and Pf liver stage antigen-3 (PfLSA3) [38].

\*Multiple epitope is a compound of known CD4+ and CD8+ epitopes from a variety of pre-erythrocytic antigens.

## Blood-stage vaccine candidates in efficacy testing

The Apical Membrane Antigen 1 (AMA1) is a polymorphic

protein expressed on the surface of both merozoites and sporozoites. AMA1 is believed to be involved with merozoites reorientation prior to erythrocytic invasion and may contribute to erythrocyte binding [39]. It has been employed in various vaccine formulations using both protein-in-adjuvant and viral vector design models. One formulation investigated is the AMA1-C1 vaccine which contains equal parts of analogous sequences of the AMA1 protein from two divergent *P. falciparum* clones, FVO and 3D7, adjuvanted with alhydrogel. Falciparum Malaria Protein 2.1 (FMP2.1) is another monovalent AMA1 derivative vaccine candidate that specifically uses the AMA1 allele of the 3D7 *P. falciparum* strain expressed in *E. coli*. AMA1 has also been incorporated into a ChAd63-MVA as a single-gene prime-boost vector vaccine (ChAd63-MVA AMA1) and as a multi-gene vector vaccine combined with the Merozoite Surface Protein 1 (MSP1). The MSP1 blood-stage antigen has been incorporated as part of the ChAd63-MVA vector platform as both a single-gene vector vaccine and as part of the multi-gene vector vaccinees ChAd63-MVA MSP1 + ME-TRAP and ChAd63-MVA AMA1+MSP1. In animal trials, the use of a recombinant adenovirus-poxvirus prime boost vaccine strategy using MSP1 in preclinical animal trials repeatedly induced robust humoral and cellular responses [40,41]. A potent CD8+ T-cell and anti-MSP1 IgG response has also been characterised in rodent models against liver-stage and blood-stage parasites, respectively [42]. The Falciparum Malaria Protein 1 (FMP1)/AS02 vaccine uses an immunogenic 42kDa portion of MSP1 derived specifically from the 3D7 *P. falciparum* strain. Field investigations of immunogenicity have shown antibodies targeting the FMP1 fragment is associated with clinical protection in humans [43,44].

The Merozoite Surface Protein 3 (MSP3) vaccine attempts to exploit evidence that antibodies directed against certain conserved domains of the surface antigen interfere with merozoite invasion [45,46]. Further field evidence suggests that parasite load negatively correlates with anti-MSP3 antibody titers [47] and compared to other anti-merozoites antigen-specific antibodies MSP3 has shown the greatest consistency in both antibody response and subsequent risk reduction for clinical malaria [48]. It is suggested that the MSP3-LSP vaccine candidate operates by a similar antibody dependent cell mediated mechanism with induced anti-MSP3 IgGs inhibiting life-cycle progression within the blood stage [49].

The GMZ2 vaccine candidate is a recombinant fusion protein of the *P. falciparum* glutamate-rich protein (GLURP) and MSP3 antigen complexed with an aluminum hydroxide adjuvant. The GLURP antigen has multiple B cell epitopes and is expressed throughout the human portion of the parasite's life-cycle [50]. In regions endemic with malaria, seroepidemiological evidence demonstrates high serum levels of anti-GLURP IgG, which has been linked with decreased malaria-associated morbidity and parasite density in children aged 5 to 9 years old [51-54]. The prospective efficacy of the GMZ2 vaccine is supported by evidence that anti-GLURP and anti-MSP3 antibodies behave synergistically as part of the natural protective response [55,56]. Furthermore, it has demonstrated consistent parasitic growth inhibition in genetic variants of *P. falciparum* from geographically distinct regions suggesting that a GMZ2 vaccine could remediate challenges posed by antigenic diversity across different strains of *P. falciparum* [57].

### Multi-stage vaccine candidates in efficacy testing

Five vaccine candidates can be considered multi-stage as they encode or contain antigens from both the pre-erythrocytic stage and blood stage. The Naval Medical Research Center Multi-antigen Multi-stage Malaria vaccine, Adenovirus vectored, *P. falciparum* CSP and AMA1 (NMRC-M3V-Ad-PfCA) vaccine is a combination of two recombinant adenovirus (Ad)-derived constructs. One expresses the pre-erythrocytic stage CS protein and the other expressing the blood stage antigen AMA1, both derived from the 3D7 *P. falciparum* strain. NMRC-M3V-DNA/Adenovirus-PfCA is a prime boost vaccine using the NMRC-M3V platform with a CS and AMA1-encoded DNA vector as the priming agent and the recombinant NMRC-M3V-Ad vaccine encoding CS protein and AMA1 as the booster component. ChAd63-

MVA MSP1+ME TRAP prime-boost candidate, as noted previously, is a multi-stage vector vaccine encoding the pre-erythrocytic ME-TRAP gene and the blood-stage MSP1 gene. Finally, FFM ME-TRAP + PEV3A combines administration of a Fowl pox strain 9 (FP9)/MVA ME-TRAP heterologous prime boost vaccine with the PEV3A vaccine. PEV3A is an influenza-based vector encoding CS protein with AMA-1, which itself has also been evaluated as a stand-alone vaccine. FP9/MVA ME-TRAP prime boost has previously demonstrated a strong capacity to stimulate cell-mediated responses with a 90% decline in liver to blood inoculate [58]. The PEV3A virosome formulation has shown strong peptide-specific antibody responses when previously assessed for immunogenicity [59].

### Transmission blocking vaccine candidates

No transmission blocking vaccine candidates have yet undergone efficacy testing in human subjects.

### Methods

The WHO Rainbow Table, which represents the most complete summary of all preclinical and clinical malaria vaccine projects worldwide [60], was used to establish a baseline of those vaccine candidates targeting *P. falciparum* that have progressed to clinical trials and possibly undergone some measure of efficacy testing. Vaccinees targeting *P. vivax* were not included in this review. Once prospective vaccine candidates were identified a systematic search was performed between Nov. 13, 2015 and Dec. 8, 2015 using the Cochrane Library, the Pub Med database, the WHO Rainbow Table Reference List\* and clinicaltrials.gov.

The Cochrane Library and Pub med database were both searched using search criteria "malaria vaccinees" AND "RTS,S" OR "ChAd63-MVA" OR "ChAd63/MVA" OR "PfSPZ" OR "FMP2.1" OR "GMZ2" OR "MSP3" OR "ChAd63 AMA1" OR "NMRC-M3V". The Pub Med database was also secondarily searched using advanced search criteria „Malaria Vaccinees“[Mesh] AND (RTS[All Fields]) OR ChAd63-MVA[All Fields] OR ChAd63/MVA[All Fields] OR PfSPZ[All Fields] OR FMP2.1[All Fields] OR GMZ2[All Fields]) OR MSP3[All Fields] OR (ChAd63[All Fields] AND AMA1[All Fields]) OR (NMRC[All Fields] AND M3V[All Fields]) AND (Clinical Trial[publication type] OR "clinical trial" [Publication Type] OR "clinical trials as topic"[MeSH Terms] OR "clinical trial"[All Fields]) AND "humans"[MeSH Terms]. Following identification of clinical trials using the above search criteria, trials were further included into this review only if vaccine efficacy in human subjects was either a primary or secondary outcome objective of the respective study. Non-human efficacy trials were excluded.

The website clinicaltrials.gov was searched using keyword "malaria vaccinees" with inclusion criteria "phase II" OR "phase III" OR "phase IV" clinical trials. It was also searched using specific vaccine names identified from the WHO Rainbow Table. For those vaccine candidates identified using clinicaltrials.gov with the project status of either "complete" or "unknown" that explicitly list evaluation of efficacy measures as a primary or secondary outcome, but with no available published results, personal communication with lead investigators was attempted.

Vaccine titles were also searched individually in all three databases. No publication date inclusion criteria were incorporated during database searches; however, final analysis focused strictly on those articles published between 2005 and present. This was designed as an attempt to focus on those vaccine candidates who are currently shaping the R&D landscape for malaria vaccinees against *P. falciparum* and to exclude defunct candidates no longer actively being investigated.

*\*The WHO Rainbow Table Reference List is a comprehensive list of citations of published articles from malaria vaccine projects globally, as originally identified from the WHO Rainbow Table.*

### Results

Following the advanced search criteria detailed above a total of 301 trials were identified in the Cochrane Library, 152 in the Pub

Med Database and 327 at [clinicaltrials.gov](http://clinicaltrials.gov). Of these, only 21 clinical trials were subsequently identified and included in this review based on the inclusion criteria that vaccine efficacy in human subjects was evaluated as either a primary or secondary outcome objective and trial results were published in 2005 or after.

### Pre-erythrocytic vaccine candidates

Two clinical studies were identified for the *Pf*SPZ vaccine. When irradiated *Pf*SPZ was administered either subcutaneously or intradermally it failed to induce significant clinical protection or immune response following CHMI with only 4.5% of immunised volunteers demonstrating sterile immunity following CHMI challenge [61]. No further statistical analysis was provided. Alternatively, by administering multiple doses of  $1.35 \times 10^5$  *Pf*SPZ intravenously, 100% of vaccinees receiving five doses ( $p = 0.015$ ) and 66% receiving four doses demonstrated sterile immunity pursuant to CHMI [62]. Ultimately, vaccinees who received a  $1.35 \times 10^5$  *Pf*SPZ dose regimen demonstrated an overall sterile efficacy of 80% ( $p = 0.028$ ). The three vaccinees who received four doses of  $1.35 \times 10^5$  *Pf*SPZ but subsequently tested positive on blood smear for *P. falciparum*, did however demonstrate a delayed time between sporozoite challenge and PCR confirmation of parasitemia compared to the median control time. However, no statistical analysis was provided to confirm significance. Lower dose regimens of *Pf*SPZ vaccine administered intravenously showed no significant protection upon challenge. However, of the nine volunteers receiving four doses of  $3 \times 10^4$  *Pf*SPZ, eight demonstrated a 1.4-day delay to a positive blood smear for parasitemia compared to the controls subjects ( $p = 0.007$ ). No other low dose regimen cohort demonstrated significant delays to parasitaemia.

Although myriad studies have been published evaluating the efficacy data reported from the phase III trials of RTS,S/AS01, only three reports specifically evaluated efficacy outcomes as a primary outcome measure. In all three studies efficacy was measured using cases of clinical malaria per person-year, compared to unvaccinated control cohorts [63–65]. For infants (aged 6 to 12 weeks), the observation period was over a median 38 month period while for children (aged 5 to 17 months) the median observation period was over 48 months. Outcomes were more significant in children, compared with infants. In patients who received a three RTS,S dose regimen (per-protocol) with no booster shot, vaccine efficacy against all cases of clinical malaria was 32.9% (95% CI: 26.3% - 38.8%) in infants and 55.1% (95% CI: 50.9% - 59.3%) in children. Vaccine efficacy against severe malaria was 36.6% (95% CI: 4.6% - 57.7%) in infants and 47.3% (95% CI: 22.4% - 64.2%) in children. For patients who received at least a single dose of RTS,S but did not complete per-protocol dosing (intention-to-treat), vaccine efficacy against clinical malaria was 30.1% (95% CI: 23.6% - 36.1%) in infants and 50.4% (95% CI: 45.8% - 54.6%) in children, while vaccine efficacy against severe malaria was 26.0% (95% CI: -7.4% - 48.6%) in infants and 45.1% (95% CI: 23.8% - 60.5%) in children. Vaccine efficacy was not consistent over the study period and declined with time in both age categories. Vaccine recipients who received a booster shot of RTS,S at 20 months demonstrated an added overall efficacy of 25.6% (95% CI: 18.2% - 32.3%) over the subsequent 12 months versus immunised controls who did not receive a RTS,S/AS01 booster.

When ChAd63-MVA CS prime-boost was administered intramuscularly followed with CHMI, only 7% of volunteers demonstrated sterile immunity, failing to achieve statistical significance ( $p = 0.18$ ) [35]. Of the 14 vaccinees that developed infection, three demonstrated a delay to treatment compared to controls. ChAd63-MVA CS did not demonstrate any significant reduction in mean parasite density following *P. falciparum* challenge ( $p = 0.08$ ).

In evaluating ChAd63 ME-TRAP, two of fifteen volunteers who received the vaccine developed sterile immunity to the controlled *P. falciparum* challenge [35]. However, no statistically significant efficacy was demonstrated ( $p = 0.09$ ). Five of fifteen vaccinees demonstrated

a delay in time to treatment compared to controls without statistical significance ( $p = 0.09$ ). That said, a significant reduction in mean parasite density ( $p = 0.01$ ), as measured using qPCR, was seen at 7.5 days following *P. falciparum* challenge. An alternative study found that 21.4% ( $p = 0.008$ ) of ChAd63-MVA ME-TRAP vaccinees demonstrated sterile immunity following controlled challenge, while 36% ( $p = 0.008$ ) of those who did develop infection showed a delayed time to symptoms [66]. Of those vaccinees who demonstrated sterile immunity following initial *P. falciparum* challenge, one volunteer maintained sterile protection while another demonstrated a delay to patent parasitaemia when each was re-challenged eight months later. None of the vaccinees who received the ChAd63 ME-TRAP vaccine without the MVA-ME-TRAP booster were protected against infection following challenge. In field trials of ChAd63-MVA ME-TRAP prime-boost undertaken in Kenya, the vaccine demonstrated a 67% (95% CI: 33% - 83%,  $p = 0.002$ ) reduction in risk to clinical malaria in adults using PCR-analysis [67]. Alternatively, when parasitemia  $> 10$  parasites/ml blood was assessed as a secondary efficacy measure, vaccine efficacy was reported to be 82% (95% CI: 46% - 94%,  $p = 0.002$ ).

Finally, evaluation of MuStDO5 found that following CHMI no volunteers who received the vaccine demonstrated sterile protection with no significant delay in parasitaemia compared to controls ( $p = 0.08$ ) [68].

### Blood-stage vaccine candidates

No vaccinees who received either ChAd63-MVA AMA1 or ChAd63-MVA MSP1 developed sterile immunity following *P. falciparum* challenge [69]. No statistically significant efficacy or delay in time to parasitaemia was observed for either vaccine (AMA1  $p = 0.28$ ; MSP-1  $p = 0.13$ ). One of nine recipients of ChAd63-MVA AMA1+MSP1 developed sterile immunity following challenge; however, no statistically significant efficacy was measured ( $p = 0.071$ ). In an unspecified subset of patients who received ChAd63-MVA AMA1+MSP1 a delay in time to parasitaemia was observed; however, no overall delay in time to parasitaemia was reported nor was statistical analysis provided.

In field trials in Kenya, FMP1/AS01 failed to demonstrate any significant overall efficacy with a vaccine efficacy of 5.1% (95% CI: -26% - 28%;  $p = 0.7$ ) being reported [19].

No statistically significant overall efficacy ( $p = 0.18$ ) was observed following FMP2.1/AS01A administration to Malian adults [70]. Alternatively, a 64.3% (95% CI: 0.08 - 0.86,  $p = 0.03$ ) vaccine efficacy was reported when adjusted for clinical cases caused specifically by *P. falciparum* strain 3D7. Unadjusted efficacy was insignificant. This group also observed that FMP2.1/AS01A reduced blood parasite density in both intention-to-treat ( $p = 0.01$ ) and per-protocol cohort ( $p = 0.01$ ). When evaluated to assess effect on parasite multiplication rate (PMR), FMP2.1/AS01B administration intravenously was concluded to have no significant impact on time to treatment following CHMI compared to unvaccinated controls”.

Vaccination with an MSP3 vaccine candidate in a small cohort of Burkinabe children resulted in an overall reduction in incidence of infection from 5.3 clinical cases per 100 days for control subjects to 1.55 per 100 days for vaccinees ( $p = 0.01$ ) [71]. According to [clinicaltrials.gov](http://clinicaltrials.gov), another field test of MSP3 lead by the Malaria Research and Training Center in Bamako, Mali, appears to be on hiatus ([clinicaltrials.gov](http://clinicaltrials.gov) NCT00652275). Attempts to contact lead investigators failed.

AMA1-C1/Alhydrogel demonstrated no significant outcomes specific to measures predictive of potential efficacy [22]. Additionally, no significant efficacy was demonstrated with either AMA1/AS01A or AMA1/AS01B when evaluated in CHMI trials using blood smear analysis ( $p = 0.23$ ) or by qPCR ( $p = 0.19$ ) [20]. Finally, at the time this review was submitted for publication, results of a completed phase IIB efficacy trials of GMZ2 have yet to be released. Communication with the lead investigator revealed that a manuscript is currently in preparation.

## Multi-stage vaccine candidates

No volunteers were observed to develop sterile immunity following CHMI after co-administration of ChAd63-MVA MSP1 and ChAd63-MVA ME-TRAP ( $p = 0.2$ ). A single volunteer demonstrated delay to parasitaemia; however, overall there was no significant delay compared to controls [69]. Of vaccinees who received the NMRC-M3V-D/Ad-PfCA prime boost regimen, four of fifteen vaccinees demonstrated sterile immunity after challenge with *P. falciparum* with no reporting of statistical significance [72]. Of vaccinees that became infected there was no significant difference in either time to diagnosis or in parasite multiplication rate compared to controls. When NMRC-M3V-Ad-PfCA was administered without the DNA-vector prime, sterile immunity was not observed in any vaccinees following *P. falciparum* challenge [73]. One of eighteen vaccinees did demonstrate a significant delay in time to parasitaemia compared to controls; however, no overall statistically significant efficacy was measured using this marker ( $p = 0.489$ ). Similarly poor efficacy outcomes were observed with NMRC-M3V-Ad-PfC, without AMA1, where two of eleven vaccinees demonstrated a delay in time to parasitaemia but no statistically significant delay to time to parasitaemia was observed overall ( $p = 0.46$ ). Sterile immunity was not observed in any vaccine recipients [74].

Neither co-immunization with FP9/MVA ME-TRAP + PEV3A or administration of PEV3A alone showed a statistically significant delay in time to parasitaemia ( $p = 0.65$ ) [75].

*“Trial unpublished at time of this manuscript’s submission. P-values not presented in abstract obtained from corresponding author.”*

## Discussion

To date, malaria vaccine candidates targeting the pre-erythrocytic stage of the *P. falciparum* life cycle have demonstrated more substantive levels of clinical efficacy than either blood-stage or multi-stage vaccinees. RTS,S/AS01E, in particular, has demonstrated that protein-in-adjuvant subunit vaccinees can provide a reasonable degree of protection against both clinical and severe malaria through stimulation of humoral immune mechanisms. However, the levels of protective efficacy achieved by RTS,S/AS01E barely met the strategic goals of the 2006 WHO Malaria Vaccine Technology Roadmap to license a malaria vaccine with 50% protective efficacy by 2015 [76]. A goal that RTS,S/AS01E only achieved in children aged 5-17 months. Given that the revised long-term strategic goal set out in the 2013 WHO Malaria Vaccine Technology Roadmap to attain a minimum 75% vaccine efficacy is well beyond the demonstrated capacity of RTS,S/AS01, it is clear that alternative candidates need further development.

Early clinical trials of attenuated whole sporozoite vaccine candidate PjSPZ are providing impressive efficacy outcomes superior to those attained with RTS,S/AS01. Compared to earlier testing of PjSPZ, protective efficacy was drastically improved to more than 80% by altering route of vaccine administration and dosage [62]. The results highlight the significant potential of the attenuated whole-parasite vaccine model. The ChAd63-MVA ME-TRAP vaccine also demonstrated strong protective capacity particularly when evaluated in field trials, compared to previous outcomes from controlled trials.

Although the rationale for such inconsistency is unknown, it may in part be due to exposure heterogeneity in field versus controlled trials. That is, increased heterogeneity as seen with field trial testing leads to fewer vaccine subjects being exposed to the parasite in comparison to controlled trials where all trial volunteers receive a standardized exposure [77]. Controlled trials become more susceptible to underestimating vaccine efficacy, which may explain the inconsistent efficacy observations of ChAd63-MVA ME-TRAP. This highlights the limitation that although field trials using naturally acquired malarial infections are imperative for evaluating protective efficacy with real-world application, the inherent heterogeneity in transmission rates may skew comparative analysis when attempting to assess the relative rates of efficacy demonstrated by various vaccine candidates [30].

Blood-stage vaccine candidates have in general failed to achieve reasonable efficacy outcomes even though they have repeatedly demonstrated capacity to induce immune responses. Due to likely numerous unknown reasons immunogenicity has not translated to clinical protection. It could possibly be due to the limited role any single antigen plays in the overall story of blood-stage immunity or perhaps an inappropriate selection of antigen for vaccine use. On these points, all malarial vaccinees may in fact be self-limiting in that compared to our natural response to malarial infection, they fail to leverage the absolute immunogenic properties of multiple antigenic targets and the synergistic response this likely creates in the natural immune response. Another challenge which has affected at least one blood-stage vaccine, and possibly others, is antigenic polymorphism. When efficacy was adjusted for 3D7 *P. falciparum* strain-specific AMA1, FMP2.1/AS01A demonstrated a statistically significant protective capacity while unadjusted efficacy data showed no overall protection [70]. Given the inherent shortfall observed due to allele-specificity of this vaccine target, it brings to light the necessity to identify and incorporate highly conserved antigenic targets into future vaccine designs, which could prove difficult. Effects of polymorphism have also been reported with RTS,S with evidence suggesting that vaccine efficacy among children 5 to 17 is dependent on the distribution of allelic variants of CS protein in the local parasite population that match the allele type employed in vaccine design [78]. This again highlights a likely shortcoming of our current design models.

Multi-stage, multi-antigen vaccinees provide the benefit of targeted interference at more than one stage of parasite development. It is suggested that by activating multiple immune mechanisms, combination vaccinees should enhance vaccine immunogenicity. For example, a vaccine designed to block both the transmission and blood stages can provide protection against clinical malaria by preventing merozoite invasion of erythrocytes, while also limiting exposure to the parasite and thus risk of infection by blocking transmission. One such pre-clinical candidate combines gametocyte antigen Pfs48/45 with blood-stage antigen GLURP. In murine models to date, this candidate has demonstrated strong immunogenicity compared to baseline measures of either vaccine alone [79]. In addition to the direct immunogenic advantages of inducing a synergistic multi-faceted immune response, a multi-antigen approach model could help side-step challenges related to antigenic polymorphism and strain diversity as discussed above. This approach could also help minimize barriers presented by genetically-restricted immune responses, such as the challenge of inducing a potent CD8+ T cell response by a blood stage vaccine [80].

Interestingly to date, multi-stage malarial vaccinees have failed to convert immunogenic potential into any substantive clinical protection in early efficacy testing. Only NMRC-M3V-D/Ad-PfCA prime boost has demonstrated modest statistically significant efficacy. However, the predictive strength of these outcomes in large-scale application remains hampered by the limited sample size and observation periods used during evaluation. The results confirm proof-of-concept for NMRC-M3VD/Ad-PfCA, but understanding the long-term potential efficacy of this vaccine will require further field testing. Unlike the NMRC-M3V-D/Ad-PfCA vaccine, NMRC-M3V-Ad-PfCA was unable to provide sterile protection or delay time to parasitaemia, suggesting that the DNA-vector is necessary to prime an initial immune response for subsequent exposure to the adenovirus plasmid.

Future design modeling of malaria vaccinees will likely investigate mechanisms to further potentiate combined humoral and cell-mediated immune responses as means to increase protective efficacy, as employed with heterogeneous prime-boost models. Although this approach more closely reflects the body’s own natural defences to *P. falciparum*, demonstrated efficacy remains elusive [81-83]. Unlike the homologous prime-boost approach which is additive in nature, the heterologous prime-boost mechanism encourages a synergistic response capable of stimulating a substantial cellular response and improved efficacy [84-86]. That said, a number of heterologous prime-boost malaria vaccine candidates, including ChAd63-MVA MSP1, ChAd63-MVA AMA1 or ChAd63-MVA MSP1+ME-TRAP have failed to convert potent cellular stimulation into clinical protection.

Given the track record of the heterologous prime-boost strategy as a vaccine model against other common pathogens, this may be the result of inappropriate antigen selection or dosing regimen, for example, rather than a failure of the prime-boost model itself. Alternatively, the immunologic effect of homologous prime boost involves induction of a potent humoral response, with only secondary cellular activity [30]. It has been posited that this may prevent antigen presentation and cell-mediated activity long-term and in effect may inherently limit a vaccinees efficacy [34].

Heterologous prime-boost using a gene-vector priming agent with a protein-in-adjuvant boosting agent is being evaluated as a potential model to induce strong cellular and humoral responses. Preclinical trials of a combination ChAd63-MVA ME-TRAP and RTS,AS01 vaccine has shown enhanced protection in animal models through induction of robust humoral and cellular immunity [87,88]. Predictive modeling of a combination ChAd63-MVA ME-TRAP and RTS,S/AS01 vaccine suggests that such a candidate could achieve significant levels of sterile immunity in human subjects [89]. Unfortunately, the model provides no insight into the long-term protective efficacy or immunogenicity of the vaccine.

Aside from RTS,S/AS01, the long-term protective efficacy of other vaccine candidates has yet to be fully evaluated through late stage clinical trials. No candidates have demonstrated lasting protection, including RTS,S/AS01 itself, which gradually loses its immunogenicity and efficacy over a period of months [12,65]. Additionally, besides RTS,S/AS01 most clinical trials identified by this review observed volunteers for no more than six months following exposure to *P.falciparum*. For the studies using CHMI, volunteers were only challenged a single time which impairs analysis of long-term protection to multiple exposures. Ultimately, the capacity of a vaccine to induce a strong protective effect becomes nullified if the effect wanes quickly with time as there is limited practicality in developing and licensing a malaria vaccine which requires multiple primer doses, including multiple booster doses. Considering that regions most affected by malaria are often health-resource poor, pursuing a universal immunization campaign with a vaccine requiring a complex dosing regimen creates significant logistical challenges. Moving forward, it is important that protective efficacy therefore not only be defined by the marker used to assess efficacy, but also by the duration with which measured efficacy remains intact.

Development of a clinically effective malaria vaccine that can achieve the efficacy targets outlined in the 2013 Malaria Vaccine Technology Roadmap remains in relative infancy. The success of RTS,S/AS01 is most certainly a cause for celebration; however, the vaccinees reported efficacy measures should not be the sole recipient of such accolades. Instead, the true success of RTS,S/AS01 is that it has established a precedent from which to build upon. It serves as confirmation that a malarial vaccine is possible and warrants further investment to maximize efficacy outcomes while using dosing regimens considerate of the health-resource realities of endemic regions. To date, pre-erythrocytic vaccine candidates have demonstrated superior protective efficacy compared to early trials of blood-stage and multi-stage vaccinees. The pre-erythrocytic stage is an important vaccine target as it represents a bottleneck in the parasite's life-cycle and directly impacts transition to blood-stage infection. However, best evidence suggests that blood-stage, transmission stage, and combination design models alongside continued innovations in vaccine delivery will serve important roles in improving efficacy measures with further development.

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## Appendix Files

[Appendix File 1](#)

[Appendix File 2](#)

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