MALARIA

Challenges and strategies for developing efficacious and long-lasting malaria vaccines

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Although there has been major recent progress in malaria vaccine development, substantial challenges remain for achieving highly efficacious and durable vaccines against *Plasmodium falciparum* and *Plasmodium vivax* malaria. Greater knowledge of mechanisms and key targets of immunity are needed to accomplish this goal, together with new strategies for generating potent, long-lasting, functional immunity against multiple antigens. Implementation considerations in endemic areas will ultimately affect vaccine effectiveness, so innovations to simplify and enhance delivery are also needed. Whereas challenges remain, recent exciting progress and emerging knowledge promise hope for the future of malaria vaccines.

INTRODUCTION

Malaria remains as one of the world's leading health issues, responsible for >200 million clinical cases and up to 500,000 deaths annually, and is a leading cause of death among young children (*1*). The strong need for malaria vaccines with high efficacy to achieve control and elimination is recognized by key global organizations. This need is heightened by the spread of antimalarial drug and insecticide resistance (*1*), recent rebound increases in malaria in many regions, and stalled progress toward reducing the global malaria burden (*1*). The World Health Organization (WHO) and partners set a strategic goal of developing malaria vaccines with >75% efficacy against clinical malaria and suitable for use in all malaria-endemic areas by 2030 (duration of protection demonstrated over ≥2 years and booster doses required no more frequently than annually) (*2*).

However, achieving vaccines with sustained high efficacy has been an enduring challenge. Animal models have provided a proof of concept for various experimental vaccines, but very few have shown strong efficacy in human clinical trials. While multiple *Plasmodium* spp. can cause human malaria, *Plasmodium falciparum* is responsible for most of the cases and deaths, particularly in Africa, and has been the major focus of vaccine development. *Plasmodium vivax* is the second major cause of malaria, but few vaccines have been developed and tested for this species, in part, because of the relatively recent recognition of the severe forms of this infection and because of the challenges in maintaining *P. vivax* in culture in vitro. Other species—*Plasmodium knowlesi*, *Plasmodium ovale*, and *Plasmodium malariae*—are responsible for a small burden of disease and are not currently a major focus of vaccine development.

The complexity of malaria is a major challenge for vaccine development; with over 5000 genes, there are hundreds of potential targets and vaccine candidates over different life stages (Fig. 1). Vaccine strategies can be broadly grouped by the malaria parasite life stage they target. Pre-erythrocytic vaccines target sporozoites, which are inoculated by a mosquito and travel to the liver to initiate infection in hepatocytes, and/or parasite-infected hepatocytes. Pre-erythrocytic vaccines are attractive because they can prevent initial infection and thereby prevent clinical illness and malaria transmission. After replication in hepatocytes, merozoite forms are released into the blood where they infect red blood cells (RBCs) and undergo asexual replication, releasing new merozoites that infect RBCs. Blood-stage vaccines generally target the merozoite form and aim to prevent replication and the development of clinical illness. Reducing blood-stage parasitemia may also reduce the transmission of malaria. Transmission occurs when some intraerythrocytic parasites differentiate into sexual forms, known as gametocytes, which can be taken up by mosquitoes where they undergo sexual replication, and are subsequently transmissible to humans. Transmission-blocking vaccines (TBVs) target gametocytes and parasite stages in the mosquito midgut to prevent infection of mosquitoes. However, the success of this strategy has not yet been demonstrated in clinical trials or human population studies.

CURRENT STATE OF MALARIA VACCINES TESTED IN CLINICAL TRIALS

After successful preclinical development, numerous approaches have been used to evaluate vaccines for efficacy in clinical trials, using different outcome measures. Demonstrating efficacy against malaria in children under conditions of natural exposure is required, but early clinical-phase trials may first test for efficacy in preventing infection or clinical illness or reducing parasitemia in adults in endemic regions. A small number of vaccines have shown efficacy in phase 2 trials. However, in general, efficacy has been modest and has lacked sustained protection. Because of the cost, complexity, and size of phase 2 field trials, there has been an increasing use of human challenge models whereby vaccinated adults undergo controlled human malaria infection (CHMI), delivered via mosquito bite or intravenous injection. While many candidate vaccines have been evaluated in preclinical models, we will focus our review on those vaccines that have been tested for efficacy in human trials (Table 1).

Pre-erythrocytic vaccines

RTS,S is the most advanced malaria vaccine candidate and is based on a virus-like particle (VLP) containing central repeat and C-terminal

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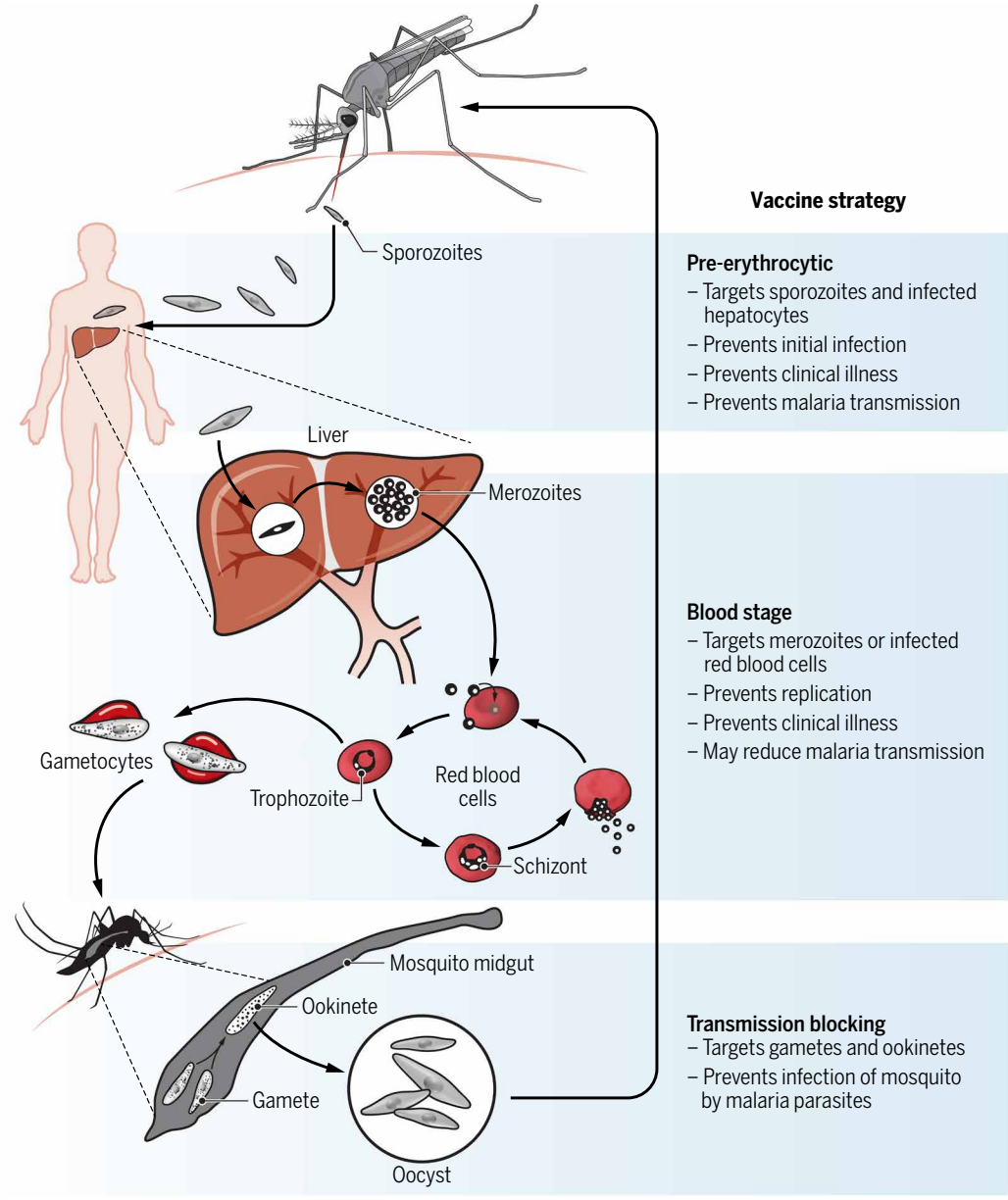

Fig. 1. *Plasmodium* life cycle and targets for vaccine development. Different life cycle stages of *Plasmodium*, the protozoan parasite that causes malaria. These stages include sporozoites that infect hepatocytes and develop inside them (pre-erythrocytic stages), merozoites that infect RBCs and develop into trophozoites and schizonts (blood stages), gametocytes that develop inside RBCs and are taken up by female mosquitoes during a blood meal, and gametes and ookinetes that develop in the mosquito gut (transmission stages). The parasite target antigens of the three major types of vaccines—pre-erythrocytic, blood stage, and transmission blocking—are indicated in Table 2. Pre-erythrocytic vaccine candidates target sporozoites in the blood or developing parasites in hepatocytes. Blood-stage vaccines predominantly target merozoites, which are extracellular for a short time after release from the liver or schizont before they invade erythrocytes, but some target the parasite antigen PfEMP1 expressed on the surface of infected erythrocytes. TBVs induce antibodies against the transmission forms of the *Plasmodium* parasite, the gametes and ookinetes, to prevent infection of mosquitoes and subsequent transmission of sporozoites to another human host.

epitopes of the major sporozoite surface antigen, circumsporozoite protein (CSP), with the aim of generating immunity that would prevent infection and subsequent malaria. A series of promising phase 1 and phase 2 trials led to a large phase 3 trial of >15,000 infants and young children across 11 sites in sub-Saharan Africa. In children 5 to 17 months old at first vaccination, vaccine efficacy assessed over 12 months of follow-up was 50.4% (95% confidence interval, 45 to 54%; intention-to-treat analysis) and vaccine efficacy against severe malaria was 45.1% (95% confidence interval, 23 to 60%). Among infants 6 to 12 weeks of age, vaccine efficacy was 30.1% (95% confidence interval, 23 to 36%). Over 3 to 4 years of follow-up, vaccine efficacy was 28 and 18% without booster, in children and young infants, respectively, and 36 and 26% in those who received a booster dose at ~18 months (*3*). A key limitation with RTS,S is waning of vaccine efficacy over time, and therefore, identifying strategies to generate more sustained efficacy is a priority. Statistical analyses suggested that vaccine efficacy was higher initially (60 and 40% at 3 months for the children and infants, respectively) but quickly waned to limited efficacy by 18 months (*4*). There was high variability in vaccine efficacy between study sites over the 18-month follow-up, ranging from 40 to 77% among children and 0 to 49%

Table 1. Overview of vaccines tested for efficacy in clinical trials. PCR, polymerase chain reaction; spz, sporozoite.

Vaccine	Phase; schedule	Participants*	Efficacy % (95% CI)†,‡,§	Reference
RTS,S/AS02	2b; 0-1-2 months	1–4 years, Mozambique ($n = 746$)	2–8 months: 30(11–45)† 8–21 months: 29(8–45)† 21–33 months: 17(−3–32)† 33–45 months: 12(−20–35)†	(*148*)
	1/2b; 0-1-2 months	10 weeks, Mozambique ($n = 94$)	2–6 months: 66(43–80)† 3–14 months: 33(−4–57)†	(*149, 150*)
	2b; 0-1-2 months	8 weeks, Tanzania ($n = 159$)	2.5–7 months: 59(−2–83)† 2.5–20 months: 35(−9–61)†	(*151, 152*)
RTS,S/AS01	2; 0-1-2 months	5–17 months, Kenya ($n = 241$)	0–1 year: 46(21–63)‡ 1–2 years: 25(−19–53)‡ 2–3 years: 22(−17–48)‡ 3–4 years: −1(−47–31)‡	(*153*)
	2; a) 0-1-2 months b) 0-1-7 months	6 weeks, Ghana, Tanzania, Gabon (a, $n = 166$; b, $n = 165$)	a) 2–14 months: 57(33–73)‡ b) 2–19 months: 32(16–45)‡	(*114*)
	3; a) 0-1-2 months b) 0-1-2-20 months	Sub-Saharan Africa, 5–17 months (a, $n = 2468$; b, $n = 2444$), 6–10 weeks (a, $n = 1837$; b, $n = 1824$)	a) 0–48 months: 5–17 months, 28(23–33)‡ 0-36 months: 6–10 weeks, 18(12–24)‡ b) 0–48 months: 5–17 months, 36(32–41)‡ 0–36 months: 6–10 weeks, 26(20–32)‡	(*154*)
PfSPZ	1/2a; 1.3×10^5 spz a) 4 doses b) 5 doses	Malaria-naïve adults (a, $n = 9$; b, $n = 6$)	a) 67§ b) 100§	(*11*)
	1/2a; 9×10^5 spz, 3 doses	Malaria-naïve adults ($n = 14$)	64§	(*13*)
	1/2a; 2.7×10^5 spz, 5 doses	Malaria-naïve adults ($n = 13$)	92§	(*12*)
	1/2; 2.7×10^5 spz, 5 doses	18–35 years, Mali ($n = 41$)	First blood smear positive, 0–24 weeks: 29	(*14*)
	1/2; 5 doses a) 1.35×10^5 spz b) 2.7×10^5 spz	18–35 years, Tanzania (a, $n = 18$; b, $n = 20$)	a) 6§ b) 20§	(*16*)
PfSPZ-CVac	1/2a; 3-dose spz a) 3.2×10^3 b) 1.28×10^4 c) 5.12×10^4	Malaria-naïve adults ($n = 9$ all groups)	a) 33§ b) 67§ c) 100§	(*18*)
ChAd 63 ME-TRAP	1/2a; 0-56 days	Malaria-naïve adults ($n = 15$)	13§	(*155*)
	2b; 0-56 days	18–50 years, Kenya ($n = 61$)	PCR positive 0–119 days: 66(31–83)	(*8*)
PvCSP(VMP0001)AS01B	1/2a; a) 0-4-12 weeks, low dose b) 2-6-12 weeks, medium dose c) 4-8-12 weeks, high dose	Malaria-naïve adults (a, $n = 9$; b $n = 8$; c, $n = 10$)	0 for all groups§	(*10*)
AMA1-C1/alhydrogel	2b; 0-28 days	2–3 years, Mali ($n = 142$)	0	(*21*)
AMA1-C1/alhydrogel + CPG7909	1/2a	Malaria-naïve adults ($n = 5$)	0§	(*156*)
FMP2.1(AMA1)/AS02A	2b; 0-1-2 months	1–6, Mali ($n = 186$)	0–8 months: 17.4(−8.9–37.4)† 0–24 months: 7.6(−16.7–26.8)†	(*20*)
FMP2.1(AMA1)	1/2a; 0-1-2 months a) AS01B b) AS02A	Malaria-naïve adults (a, $n = 6$; b, $n = 10$)	0§	(*157*)
FMP2.1(AMA1)/AS01	1/2a; 0-28-56 days	Malaria-naïve adults ($n = 12$)	0§	(*158*)

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Vaccine	Phase; schedule	Participants*	Efficacy % (95% CI)†,‡,§	Reference
CHAD63-MVA	2a; 0-56 days a) MSP1 b) AMA1 c) MSP1 + AMA1 d) AMA1 + ME-TRAP	Malaria-naïve adults (a, $n = 8$; b, $n = 9$; c, $n = 9$; d, $n = 10$)	a) 0[ɣ] b) 0[§] c) 11[§] d) 0[§]	(*24*)
$MSP1_{42}$ (FMP1/ASO2)	2b; 0-1-2 months	12-47 months, Kenya ($n = 191$)	2–8 months: 5.1(−26–28)[†]	(*23*)
Combination B (SMP1, MSP2, and RESA)	1/2b; 0-4 weeks	5–9 years, Papua New Guinea ($n = 60$)	Reduction in parasite density 8–18 weeks: 62(13–84)	(*28*)
GMZ2 (MSP3 + GLURP)	2b; 0-4-8 weeks	1–5 years, Burkina Faso, Gabon, Ghana, Uganda ($n = 868$)	Malaria episode 2–8 months: 13.6(3.6–23)	(*27*)
NYVAC-Pf7: CSP, SSP2, LSA1, MSP1, AMA1, SERA Pfs25	1/2a; 0-4-26 weeks a) low dose b) high dose	Malaria-naïve adults (a, $n = 19$; b, $n = 16$)	a) 5[§] b) 0[§]	(*86*)
PEV3A	2a; 0-4-8 weeks a) PEV3A b) PEV3A + FFM ME-TRAP	Malaria-naïve adults (a, $n = 12$; b, $n = 12$)	a) 0[§] b) 0[§]	(*159*)

*Number of participants who received all doses of test vaccine or the number who were challenged in CHMI studies. †Efficacy against first clinical episode of malaria (note that this definition varies between studies). ‡Efficacy against all clinical episodes of malaria. §Efficacy against CHMI (note that only protection against infection was included, in some studies there may have been a delay in parasitemia).

among young infants (*4*). Despite efficacy being below current desired goals, modeling studies (accounting for transmission intensity, intervention usage, vaccine coverage, and other parameters) predict that RTS,S would avert a substantial number of clinical cases and severe episodes and contribute to improving public health (*5*). However, a higher rate of meningitis and all-cause mortality in girls was reported in RTS,S-vaccinated children (*3*, *6*), but it is not known whether this was causally related to vaccination. RTS,S has received a positive scientific opinion from the European Medicines Agency and is entering large implementation trials in three African countries to further assess efficacy, safety, and operational issues.

Another vaccine approach has a viral-vector heterologous prime boost using ChAd43 and modified vaccinia ankara (MVA) viral vectors expressing TRAP antigen (which is present on sporozoites and infected hepatocytes) and multiple T cell epitopes (ME-TRAP). This approach induced sterile protection in 21% and delayed patency in 36% of vaccinees in CHMI efficacy trials (*7*). Phase 2b trials demonstrated a 67% risk reduction in infection among Kenyan men over short-term follow-up (*8*), but no efficacy among Senegal adults (*9*). Only one vaccine against *P. vivax* has progressed to efficacy trials; a PvCSP-based vaccine showed no efficacy in a CHMI challenge (*10*).

Two whole sporozoite vaccine strategies have shown efficacy in clinical trials. The PfSPZ vaccine contains irradiation-attenuated sporozoites that invade hepatocytes but do not develop to blood stages. PfSPZ provided ~80% protection against CHMI in malaria-naïve adults against homologous strain challenge (*11*–*13*). However, it had lower efficacy against heterologous strains and was less immunogenic and less efficacious in field evaluations (*14*–*16*). Data from further efficacy trials are expected soon. PfSPZ-CVac involves inoculation with viable sporozoites in combination with antimalarial drug prophylaxis to kill parasites at early blood stages. High efficacy was seen in CHMI studies using the homologous strain (*17, 18*). Other whole sporozoite vaccines include the use of genetically attenuated parasites that are engineered to infect the liver but cannot progress to blood-stage infection and disease. These have been efficacious in mice but, to date, have only been tested for safety and immunogenicity in humans (*19*). However, the delivery of whole parasite vaccines would require different infrastructure and operational approaches to the current childhood Expanded Program for Immunization (EPI). The goal of the EPI is to provide universal access to relevant vaccines for at-risk populations for the control of infectious diseases. Currently, EPI vaccines are stored and transported in a ~4°C cold chain and administered by intramuscular or subcutaneous injection, or orally. Current live-attenuated whole sporozoite vaccines require storage and transport in liquid nitrogen vapor phase and delivery by intravenous injection, which is not supported by EPI.

Blood-stage vaccines

AMA1, a key invasion protein of merozoites, is the most studied blood-stage vaccine (Table 1). To date, only one AMA1-based clinical trial has shown efficacy: FMP2.1/AS02A phase 2b trial in Malian children demonstrated 64% efficacy against vaccine-like strains but did not give significant overall protection, presumably because of antigenic diversity (*20*). Another AMA1-based vaccine, AMA-C1, had no efficacy in Malian children (*21*). MSP1 is a highly abundant merozoite surface antigen, and antibodies against MSP1 are protective in animal models and associated with protection in some human cohort studies (*22*). However, a phase 2b trial of FMP2.1/AS02A(MSP1–42) in Kenyan children showed no significant efficacy despite inducing high titer antibodies (*23*). The combination of MSP1 and AMA1 using viral vectors also showed little efficacy in a CHMI trial (*24*). An MSP3-based vaccine elicited high magnitudes of cytophilic immunoglobulin G1 (IgG1) and IgG3 subclass responses in African children, and secondary analyses found that vaccinated children had a reduced incidence of malaria (*25*). The GMZ2 vaccine (MSP3 and GLURP) induced high magnitudes of functional antibodies in a phase 1 trial (*26*), but little efficacy in a larger trial in African children (*27*).

Another multiantigen vaccine (combination B; composed of MSP2, RESA, and a fragment of MSP1) showed promise in a phase 2b trial in Papua New Guinean children, with a 62% reduction in parasite density among a subgroup of vaccinees not pretreated with antimalarials (*28*). The protective effect appeared to be due to targeting of MSP2, and efficacy was strain specific against vaccine-like infections. The only *P. vivax* blood-stage candidate to undergo clinical trials, PvDBP-RII, recently completed a phase 1a trial (*29*) and induced antigen-specific T cell responses and antibodies that inhibited PvDBP-RII binding to its receptor.

Transmission-blocking vaccines

To date, no data on the efficacy of TBV candidates in clinical trials has been reported. Leading TBV candidates include Pfs230 and Pfs48/45, which are expressed by gametocytes in the human host, and Pfs25, exclusively expressed in the mosquito vector (zygote and ookinete stages). In phase 1 clinical trials, vaccines based on Pfs25 and its ortholog Pvs25 have shown induction of antibodies that block mosquito infection (*30*). A recent study reported that Pfs25-EPA/alhydrogel (Pfs25 conjugated to a detoxified form of *Pseudomonas aeruginosa* exoprotein A) induced functional transmission-blocking antibodies in healthy Malian adults. However, substantial antibody levels were only achieved after four doses, and antibody levels rapidly waned after the final dose (*31*). Vaccines based on Pfs230 and Pfs48/45 have entered clinical trials, but results are not yet available.

IMPROVING EFFICACY: THE FUTURE OF MALARIA VACCINES

On the basis of current results of vaccine trials, it is clear that reaching the WHO goal of a malaria vaccine with >75% efficacy in malaria-endemic populations is exceptionally challenging. Fundamental issues for achieving higher efficacy include (i) generation of highly potent functional immunity, which depends on a strong knowledge of mechanisms and mediators of protective responses; (ii) selection of the right antigens and epitopes (or combinations) that mediate protective immunity; and (iii) developing strategies to overcome immune evasion and prevent vaccine escape. Furthermore, emerging findings suggest that vaccine-induced responses are lower in malaria-exposed populations, reflected by lower vaccine efficacy than seen in malaria-naïve populations (Table 1), raising the prospect of considerable immune dysregulation in malaria-exposed populations that affects the ability to generate and maintain potent protective responses (*32*).

An important constraint to achieving higher vaccine efficacy is the lack of clear correlates of protection to inform research and development activities. As such, developing immunologic correlates of protection is a priority area in the WHO malaria vaccine technology roadmap. Data from studies of naturally acquired and vaccine-induced immunity, as well as evidence from animal models, point to the importance of both humoral and cellular immune responses in mediating protection from malaria. However, the relative importance of humoral and cellular immunity, as well as specific components of these responses, differs depending on the malaria life stage and by the antigenic target. Furthermore, the effects of humoral and cellular immunity on controlling parasite density differ in the kinetics of their effects (*33*). Antibodies, being preformed, can have an immediate effect, provided that their function and concentration are sufficient. Cellular immunity dependent on $CD4^+$ T cells typically takes 7 to 10 days to exert an effect because memory T cells need time to expand in number and then secrete effector cytokines and inflammatory molecules. Natural killer (NK) cells and γδ T cells can have a more immediate effect.

Mechanisms of humoral immunity

Antibodies have been identified as a key component in adaptive humoral immunity against multiple malaria stages (Table 2). Therefore, maximizing the induction of antibodies with strong functional activity will be crucial for enhancing vaccine efficacy. However, a key knowledge gap is the lack of defined immune correlates of protection, hampering the capacity to evaluate vaccines. Antibodies can function via multiple mechanisms, including direct inhibition (neutralization) by blocking key parasite receptor-ligand interactions, by interacting with Fcγ receptors (FcγRs) expressed on phagocytes to promote cellular uptake and degradation [opsonic phagocytosis and antibody-dependent cellular cytotoxicity (ADCC)], and by fixing C1q to activate the complement cascade to enhance neutralization and kill target cells (*22*). The functional properties of antibodies are influenced by multiple factors including isotype/subclass, epitope specificity, affinity, and glycosylation (*34*). Cytophilic IgG subclasses (IgG1 and IgG3) have the highest capacity to mediate Fc-dependent functions (*34*) and have been linked with protection in studies of naturally acquired immunity (*22*). IgG subclass profiles differ by specific antigens (*35*) and with the use of different vaccine adjuvants (*36*), which could be exploited in vaccine design, formulation, and dosing.

FcγRs are present on major effector cells including monocytes, macrophages, neutrophils, and NK cells, and their expression and functions differ between cell types and subtypes. Complement receptors are also differentially expressed across cell types, and complement fixation can enhance or mediate phagocytosis. Furthermore, the expression and function of FcγRs and complement receptors are altered by activation of immune cells, which may occur during malaria infection or other infections. Therefore, a strong understanding of the key effector mechanisms (including cells and antibodies), and the roles of specific FcγRs and complement, is essential for maximizing humoral immunity in vaccine design. Mice are commonly used as models for malaria vaccines. However, there are immunologically important differences compared to human immunity for murine IgG subclasses and their functions, FcγR types and functions and their expression on immune cells, and effector cell composition and abundance (*37*), as well as key biological differences in model *Plasmodium* spp. used in animal models. The course of infection and overall antigenic exposure also differ between human and murine malaria. This has major implications for the application of mouse models for evaluating vaccine-induced humoral immunity and highlights that mouse models should be used judiciously to address specific questions.

Naturally acquired and vaccine-induced antibodies to multiple pre-erythrocytic antigens are associated with a reduced risk of infection and malaria (*38*). This includes RTS,S vaccine–induced immunity, which has been shown to be associated with protection (*39*). Antibodies can target the sporozoite after inoculation in the skin, after entry to the blood vessel, and during arrest at the liver sinusoid before hepatocyte invasion (*40*). Direct neutralizing antibodies have been identified in mice where they immobilized sporozoites in the dermis (*41*). Antibodies can also directly inhibit *P. falciparum* sporozoite gliding in vitro and traversal and invasive forms of motility when tested at relatively high concentrations (*19*). Monoclonal antibodies (MAbs) induced by RTS,S vaccination also protected humanized

Table 2. Targets and mechanisms of acquired and vaccine-induced immunity.

	Sporozoite	Liver stage	Blood stage	Transmission blocking
Key targets*	CSP, TRAP AMA1	TRAP, LSA-1 CSP, CelTOS	MSPs (MSP1, MSP2, MSP3, MSP6, MSP7) AMA1, RAMA EBAs, PfRH, GLURP PfRIPR, CyRPA, SERA5 MSP-DBLs PfEMP1	Pfs230, Pfs25, Pfs48/45
Established humoral mechanisms†	Inhibition of hepatocyte traversal and invasion Antibody-complement activation		Inhibition of RBC invasion Inhibition of schizont rupture Antibody-complement activation Fc-receptor–mediated effector mechanisms (phagocytosis, ADCC, ADCI) Inhibition of vascular adhesion (IEs only)	Inhibition of mosquito infection Inhibition of fertilization Antibody-complement activation
Established cellular mechanisms†	$CD4^+$ T cell help‡	Lysis of infected hepatocytes by $CD8^+$ T cells $CD4^+$ T cell help for $CD8^+$ T cells	$CD4^+$ T cell help‡ Multifunctional $CD4^+$ T cell responses $CD8^+$ T cells (*P. vivax*)	$CD4^+$ T cell help for antibody generation
Leading vaccine candidates§	CSP Whole sporozoites	TRAP, CSP, CelTOS	PfRH5, AMA1 MSP2, MSP3, GLURP SERA5 PfEMP1-VAR2CSA	Pfs230, Pfs25, Pfs48/45

*Antigens included here are major targets that have been demonstrated in multiple human studies and have been shown to be targets of functional immune responses. Not all known targets are listed. Only *P. falciparum* antigens are included; however, orthologs are present in *P. vivax* for many antigens. †Summary of demonstrated mechanisms derived from human studies and animal models. ‡$CD4^+$ T cell help for generation of antibodies and induction of proinflammatory cytokines. §For the purposes of this table, leading candidates are considered to be those that have been tested in clinical trials (particularly those with demonstrated efficacy) or antigens that have substantial efficacy in a nonhuman primate model.

mice from infection (*42*). However, direct neutralizing antibodies have not been specifically associated with protection. Recent studies established that *P. falciparum* sporozoites are susceptible to antibody-complement attack, which inhibits traversal and causes cell death (*43, 44*). Complement-fixing antibodies against CSP were associated with protective naturally acquired immunity in children (*43*), and complement fixation by anti-Gal antibodies (targeting a glycan on sporozoites) appeared to play a role in vaccine-induced immunity in a mouse model (*45*). Other Fc-dependent functions of antibodies targeting sporozoites and the roles of phagocytes are relatively understudied. Opsonic phagocytosis of antigen-coated beads by a monocyte cell line was not associated with protection in a CHMI trial of RTS,S (*46*). Given the abundance of neutrophils in the blood, an investigation of their role is warranted. Furthermore, most of the studies have focused on IgG-dependent mechanisms, but recent studies have suggested that IgM induced by vaccination can play a role (*47*).

The key role of antibodies against blood stages was clearly established by passive transfer of antibodies from immune adults to children and adults with malaria, which drives parasite clearance (*48*). Protective antibodies target merozoite antigens (*22*) and antigens on the surface of infected erythrocytes (IEs) [predominantly targeting PfEMP1; (*49*)]. Antibodies to merozoites can act by directly blocking key receptor-ligand interactions to inhibit invasion of erythrocytes (*22*). However, recent studies suggested that most acquired human antibodies rely on complement recruitment for effective inhibitory activity, and this activity was associated with immunity in children (*50*). Cytophilic IgG subclasses (IgG1 and IgG3) can promote merozoite phagocytosis by monocytes, leading to their activation, and engage FcγRs on neutrophils, leading to acute respiratory burst activity (*51, 52*). Monocyte phagocytosis can lead to the secretion of molecules that inhibit parasite growth, known as antibody-dependent cellular inhibition (ADCI) (*53*). Antibodies to infected erythrocytes are thought to act by inhibiting vascular adhesion and promoting phagocytosis by monocytes (*49*), which may be enhanced by complement (*54*). Antibodies can also inhibit parasite growth in RBCs via ADCC by NK cells (*55*). Neutrophils may also play a role; however, they have been less studied. How the relative importance of specific functions can vary between antigens is not well understood. It is likely that multiple effector mechanisms are involved in blood-stage immunity because directly growth-inhibitory antibodies have not been consistently associated with protective immunity (*22*), and antibodies that promote complement activation or opsonic phagocytosis and ADCC have been correlated with protective immunity (*22*). Furthermore, some data from mouse models support important roles for FcγRs and complement (*56, 57*).

Transmission-blocking immunity is mediated by antibodies that act primarily by neutralizing the transmission stages in the mosquito midgut after being taken up in a blood meal, thereby preventing mosquito infection (*58*). MAbs to Pfs230 and naturally acquired human antibodies were shown to induce lysis of gametes in the presence of active complement (*59, 60*). However, antibodies can have transmission-blocking activity without the need for complement fixation (*60*), and there is little knowledge on how functional transmission-blocking antibodies or complement-fixing antibodies are acquired and maintained. The roles of FcγRs and innate immune

cells remain largely unexplored question but should be prioritized in future research.

Mechanisms of cell-mediated immunity

Generating potent $CD8^+$ T cell responses is important for achieving effective immunity against infected hepatocytes, and optimizing $CD4^+$ T cell responses is likely to be essential for potent and long-lasting immunity. Targeting pre-erythrocytic stages, protection induced by PfSPZ immunization in animal models is primarily mediated by $CD8^+$ T cells (*61*). $CD4^+$ T cells can also confer protection in mice (*62*) and provide help to $CD8^+$ T cells against liver stages (*63*). The importance of $CD8^+$ T cell responses in liver-stage immunity has not been clearly established in humans, likely because of the low frequency of circulating cells in peripheral blood. The best cellular correlate of protection identified for PfSPZ vaccines in humans was frequency of the Vγ9 Vδ2 subset of γδ T cells (*11*). γδ T cells, which recognize phosphoantigens produced by the *Plasmodium* apicomplast (*64*), are important sources of interferon-γ (IFN-γ), which plays a role in protection (*65*). PfSPZ-CVac also induced parasite-specific $CD4^+$ and $CD8^+$ T cells expressing cytotoxic markers CD107a and granzyme B, respectively, which were associated with protection (*66*). Polyfunctional cytokine-producing memory $CD4^+$ T cells responding mainly to sporozoites also correlated with protection after immunization with PfSPZ-CVac (*18, 66*).

RTS,S vaccination induced CSP-specific $CD4^+$ T cells secreting T helper 1 (T_H1) cytokines interleukin-2 (IL-2), IFN-γ, and tumor necrosis factor at low frequencies (*39*). Field pediatric studies showed that vaccination elicited polyfunctional $CD4^+$ T cell responses mainly confined in memory compartments (*67*), and T_H1 cytokine responses (assessed following vaccine antigen stimulation of peripheral blood mononuclear cells) were associated with malaria protection, whereas T_H2 responses were associated with risk (*68*). $CD8^+$ T cell responses are not prominent after immunization by RTS,S, although analysis is limited to peripheral blood cells.

Targeting blood stages, cellular responses provide important effector mechanisms, in addition to humoral immunity. $CD4^+$ T cells and particularly T follicular helper cells (T_{FH} cells) are key for germinal center reactions necessary for the generation of plasma cells, high-affinity antibodies, and long-lived memory B cells (*69*). In addition, secretion of IFN-γ by αβ T cells (*70*), γδ T cells (*71*), or NK cells in response to infected erythrocytes is an important effector mechanism for blood-stage immunity. Although $CD8^+$ T cells are not thought to play a direct role in killing of *P. falciparum* blood stages, recent data suggest that they do play role against *P. vivax* through major histocompatability complex I (MHC-I) expressed on infected reticulocytes (*72*).

Optimizing adjuvants, dosing, and the use of alternative vaccine platforms

One avenue that may be leveraged to improve vaccine efficacy is optimal adjuvant usage (Table 3), which is severely hampered by the limited number of approved adjuvants for human use. An ideal adjuvant should enhance immunogenicity without compromising vaccine tolerability or safety. Alum is the adjuvant with the most extensive safety record for use in human clinical trials and is commonly used in malaria vaccine trials. However, vaccines to *Plasmodium* antigens formulated in alum induce only moderate antibodies and poor cellular responses, with an overall T_H2-skewed response (*73*). For example, early studies found that RTS,S with alum was not efficacious (*74*) and was subsequently formulated with new adjuvants AS01/AS02 to improve efficacy. AS01/AS02 adjuvants target Toll-like receptor 4 (TLR4) to stimulate the production of cytokines and costimulatory molecules, and new approaches using other TLR agonists also show promise for enhancing immune responses (*36*), which are discussed below. Further understanding of immune responses required for protective immunity and the nature of responses induced by vaccination with specific adjuvants may further improve adjuvant selection in malaria vaccines.

To elicit strong $CD8^+$ T cell responses required for protection against pre-erythrocytic stages, vaccine candidates have been formulated in recombinant viruses and administered by heterologous prime-boost strategies, which have been a promising approach for the ME-TRAP vaccine (*7*). Some prime-boost strategies have also yielded improved immune responses in animal models and humans in other infections, such as HIV and influenza (*75*). Other improvements in humoral and cellular responses may be achieved with other delivery platforms or formulations, including microneedle skin patch delivery, nanoparticles, and VLPs, to improve antigen retention and uptake by lymph nodes and antigen presentation or using specific strategies for targeting antigen-presenting cells (*36*). For example, antigens in larger particles have been found to prolong antigen presentation by dendritic cells (DCs), leading to enhanced production of T_{FH} cells and antibodies (*76*). Studies with vaccines for other infections have also pointed to the potential importance of spacing intervals between vaccine doses (*75*).

Selecting antigens and epitopes

Multiple criteria need to be considered to identify and prioritize antigens and epitopes for vaccine development. These include antigen cellular location and function, abundance, polymorphisms, data from in vitro functional assays, evidence of protective associations in studies of naturally acquired immunity, and data from animal models. Vaccine antigens that have progressed in clinical trials to date have largely been proteins identified before the era of malaria genomics and proteomics. In recent years, studies have widened the search and identified numerous new targets of immunity and promising vaccine candidates (Table 2). This advance has been most notable for merozoite antigens (*77*), but additional potential candidates for pre-erythrocytic and transmission stages are now emerging. These include 6-cys domain family members (such as Pf12, Pf38, Pf41, and Pfs47), which have potential as pre-erythrocytic vaccine targets (*78*), and transmission-blocking vaccine targets (*58, 79, 80*). Pf12, Pf38, and Pf41 are also expressed during asexual blood stages (*81*) and thus have potential in multistage vaccines. PfHAP2 was recently identified as a promising transmission-blocking candidate (*82*).

Other approaches have identified key invasion ligands essential for parasite growth. This includes PfRH5, which binds the RBC receptor basigin (*83*), as a promising blood-stage candidate, and PfRH5 vaccination was efficacious in *Aotus* monkeys when using a potent adjuvant (*84*). Understanding the importance of PvDBP in reticulocyte invasion by *P. vivax* underpinned this protein as a lead vaccine candidate (*85*). Ongoing studies are revealing key protein functions that help down-select and refine candidates for accelerated development.

Once protective antigen candidates have been prioritized, combination vaccines may further improve efficacy (Fig. 2). Several current strategies have already attempted this by combining antigens that target multiple parasite stages, such as NYVAC-Pf7 (Table 1) (*86*). Proof of concept that combination vaccines may induce higher efficacy was recently shown in mouse models where antibodies targeting

Table 3. Overview of strategies for generating highly efficacious and long-lasting vaccines.

1. Increasing vaccine efficacy	
Target key epitopes	Identify functional epitopes for host cell invasion or parasite development
	Identify epitopes for antibody-mediated complement and Fc-receptor functions
	Structure-based vaccine design: Improved targeting of functional epitopes
Maximize functional antibody activity	Direct inhibition of host cell invasion of hepatocytes and RBCs
	Direct blocking of mosquito transmission
	Complement recruitment and activation: Promote enhanced blocking of host cell invasion, parasite killing or inhibition of function, enhanced phagocytosis
	FcγR interactions for opsonic phagocytosis and ADCC, involvement of monocytes, neutrophils, and NK cells
	Define optimal IgG properties for functional activity: IgG subclass, affinity, and glycosylation
Maximize T cell activity and function	$CD4^+$ T cell help for antibody generation, cytolytic $CD8^+$ T cells, B cell, and T cell memory
	Enhanced induction of cytolytic $CD8^+$ T cells against infected hepatocytes
	Optimize T_{FH} cell responses: Maximize antibodies and plasma cells and T cell effector functions
Overcome immune escape mechanisms	Polymorphisms in vaccine candidates (e.g., CSP, AMA1): Target conserved epitopes, use multiallele vaccine approaches, whole parasite vaccines
	Define key mechanisms of immunomodulation to inform vaccine strategies (e.g., enhanced DC targeting and activation, enhanced $CD4^+$ T cell, and T_{FH} activity and phenotypes)
Adjuvants, delivery systems, dosing	Achieve optimal IgG subclass and glycosylation profiles and affinity for functional activity
	Approaches for induction of optimal combined B cell and T cell responses; includes effective targeting of DCs
	Facilitate future vaccine implementation: Simple regimens, flexible timing, adaptable to controlled-temperature cold chain delivery
Host factors	Define impact of immunogenetics on vaccine responses in endemic populations
	Address comorbidities in endemic populations (e.g., malnutrition, other infections)
	Other factors (e.g., microbiome, metabolomics, genomics)
2. Generating long-lasting protective responses	
Maintenance of protective antibodies	Maximize initial induction of antibodies targeting functional epitopes
	Induction of multiple antibody functions may maintain protective effects for longer; complement recruitment may enhance antibody function
	Identify determinants of long-lived responses in clinical vaccine trials
	Induction of long-lived antibody-secreting cells: Use of TLR agonists (including combinations), adjuvant combinations, and prime-boost strategies
B cell and T cell memory	Enhanced T_{FH} induction for $CD4^+$ T cell help and memory B cells and T cells
	Targeting DCs [e.g., using TLR agonists, DC-targeting molecules (e.g., Clec9)]
	TLR agonists, vaccine adjuvant selection, prime-boost regimens
	Vaccine particles for improved and sustained antigen presentation
	Boosting by malaria exposure: Inclusion of antigens or epitopes that boost with exposure

sporozoites (CSP) and a transmission-blocking candidate (ortholog of Pfs25) acted synergistically to reduce parasite density in a multi-generation population model (*87*). Combining multiple antigens against a single parasite stage may also result in increased efficacy, which is supported by modeling studies (*88*). Furthermore, human cohort studies have found that antibodies to multiple merozoite

antigens have higher associations with protection than single-antigen responses (*77, 89*). The breadth of the antibody response also appears important in protection induced by irradiated sporozoite vaccination (*90*). Whole parasite vaccines are an additional strategy for presenting multiple antigens in their native conformation.

Induction of protective antibodies may also be improved by identifying key epitopes targeted by protective responses. Current vaccine strategies to whole antigens or whole parasites typically consist of a range of protective and nonprotective epitopes. Refining vaccine immunogens and responses to predominantly target key functional or protective epitopes and response types may enable protective immunity to be better maintained as the overall immune response wanes. This concept underlies the rationale behind structure-based vaccine design, whereby knowledge of antigen structures and functional epitopes targeted by protective antibodies may enable enhanced immunogen design. For example, a recent study isolated antibodies from Tanzanian volunteers immunized with PfSPZ-Vac that bound to both the CSP NANP-repeat region (included in the RTS,S vaccine) and a peptide at the N-terminal junction. These antibodies were more effective at inhibiting sporozoite infection of hepatocytes in a humanized mouse model than antibodies targeting only the NANP region (*91, 92*). This suggests that inclusion of the N-terminal junction peptide in RTS,S may improve vaccine efficacy. Similarly, epitopes of inhibitory MAbs have been identified for several merozoite vaccine candidates (PfRh5, EBA175, PfAMA1, and PvDBP). However, translation of knowledge of functional epitopes into more efficacious vaccines has not yet been achieved.

Overcoming evasion of vaccine-induced immunity

Polymorphisms in antigens included in vaccines facilitate immune escape, which has been a problem in several vaccines including RTS,S and vaccines based on MSP2 and AMA1; vaccine efficacy was higher against infection of vaccine-like strains compared to vaccine-dissimilar strains (*93*). Therefore, implementation of RTS,S could shift the burden of malaria to vaccine-escape strains over time, decreasing its effectiveness. This emphasizes the need for next-generation vaccines that induce more potent cross-strain protective responses. Strategies to address this include the inclusion of multiple alleles of an antigen [e.g., AMA1 (*94*)], combinations of different antigens (*95*), or whole parasite vaccines. Antibodies induced in a phase 1 trial by

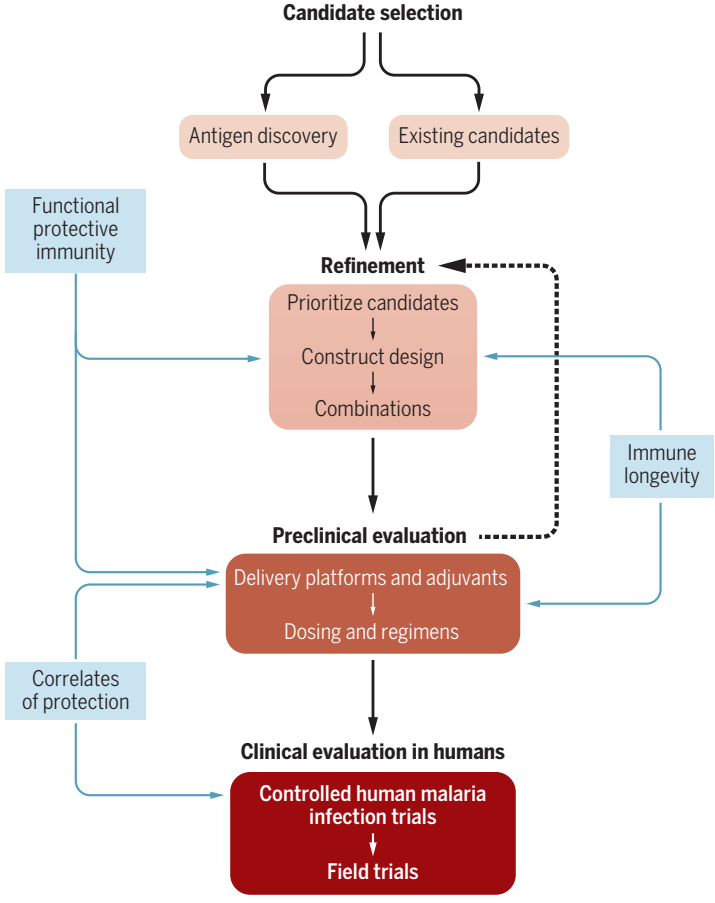

Fig. 2. Strategies to develop efficacious and long-lasting malaria vaccines. Schematic representation of key steps and activities in developing future malaria vaccines that have a high level of efficacy and generate long-lasting immunity. Potential candidate antigens for vaccine development include existing candidates and new antigens being identified from candidate discovery research. Refinement of lead candidates for development involves initial down-selection of candidates, refining immunogen design, overcoming issues of antigen polymorphism and vaccine escape by the parasite, and facilitating long-lasting immunity. This may require combinations of antigens. Preclinical evaluation (in vitro and in animal models) tests vaccine efficacy and duration and identifies strategies for optimal induction and maintenance of protective responses. Clinical evaluation of vaccine efficacy can be conducted in CHMI trials and in population-based clinical trials. Throughout this process, defining mechanisms of functional protective immunity and immune longevity and how to generate sustained immunity is crucial. Establishing correlates of immunity will greatly facilitate evaluation of candidate vaccines and aid in vaccine design and refinement.

immunization with two different MSP2 alleles surprisingly shifted antibody targeting to conserved epitopes, in contrast to naturally acquired antibodies that are overwhelmingly allele specific (*96*).

Another approach is to focus on conserved epitopes or less polymorphic antigens. Conserved epitopes have been reported in CSP, and potentially improved targeting of these by vaccination may yield higher efficacy. PfRH5 is an example of a less polymorphic antigen, and antibodies to PfRH5 have cross-strain activity in vitro (*97*). Modified vaccine antigens for hepatitis C virus and influenza have shown promising cross-strain neutralization activity (*98, 99*).

Parasite-mediated immunomodulation poses an additional challenge to vaccine development for at-risk populations. Previous malaria exposure may hamper the induction of vaccine responses, evidenced by the variation in the vaccine efficacy of RTS,S among populations with different intensities of malaria exposure (*4*), and the reduced immunogenicity and efficacy of PfSPZ vaccines in malaria-exposed populations (*14*, *15*). Specifically relevant to vaccine efficacy, DCs (the only antigen-presenting cells with the capacity to activate naïve T cells) are functionally compromised and undergo substantial apoptosis during clinical malaria and subpatent *P. falciparum* and *P. vivax* infection (*100*–*102*). Both malaria species may also induce changes to T_{FH} cells that may impair B cell help (*103*). Specifically, parasite-driven inflammation and IFN-γ production appears to skew T_{FH} development to T_H1-like subsets (*104*), which have reduced capacity to activate naïve B cells (*105*). These changes to T_{FH} subsets may be linked to the induction of atypical memory B cells that have impaired function, which may be an important constraint on the development of vaccine-mediated immunity (*106*). Changes to CD4 T cells during *Plasmodium* infection results in increased IL-10–producing CD4 T cells (*107*), which is linked to the induction of immunoregulatory networks. In some cases, specific immunosuppressive pathways induced in malaria are also found in cancer, and in the future, it may be possible to target these to modulate responses and improve vaccine efficacy [reviewed in (*108*)]. Other parasite-mediated cellular responses that may affect vaccine-induced immunity include changes to γδ T cells (*109*) and induction of trained innate immunity (*110*). Immunomodulation driven by blood-stage parasites may also disrupt pre-erythrocytic immunity (*111*).

Improving RTS,S vaccine efficacy

Given the demonstrated efficacy of RTS,S in young children, investigating strategies to improve efficacy and durability is a priority. Recent modeling studies suggest that increasing initial efficacy may have a more substantial decrease in childhood clinical cases and higher public health impact than increasing durability (*112*). New data are emerging that modifying the RTS,S vaccine schedule may improve efficacy. A regimen with a fractional (reduced) third dose given at 7 months rather than a full dose at 2 months was more efficacious in a CHMI phase 1/2a trial in malaria-naïve adult volunteers and generated increased antibody avidity and B cell somatic hypermutation (*113*). However, a previous study in infants found that a 0-2-7-month schedule (full dose) had lower efficacy than a 0-1-2-month schedule (*114*). Recently, a modified RTS,S-like vaccine construct was generated (R21), which, unlike RTS,S, does not require excess hepatitis B surface antigen expression for VLP formation. Initial studies suggest that this generates higher magnitudes of antibodies and showed efficacy in preclinical studies in mice (*115*).

Other strategies could include adding additional alleles of CSP, because polymorphisms affect vaccine efficacy (*93*) including additional regions of CSP (*91*, *92*), or adding additional antigens. Different adjuvants may generate an IgG subclass profile with greater functional activity. The lack of an established correlate of protection and incomplete understanding of the mechanisms of immunity remain a substantial impediment to advancing RTS,S efficacy. Systems biology approaches have been useful in identifying factors that predict responses with several existing vaccines and have recently been applied to identify transcriptional features related to RTS,S immune responses (*116*). Furthermore, understanding the potential impact of host factors on vaccine immunogenicity and basis for the wide diversity in vaccine responses seen among vaccinated children would enable approaches to generate more consistent protective responses and improved boosting. In addition to previous malaria exposure and immunomodulation, these may include nutrition, microbiome, and genetic factors.

GENERATING LONG-LASTING IMMUNITY

Identifying and developing strategies to generate more durable and long-lasting protective immunity is an additional high priority (Table 3). All malaria vaccines to date have shown relatively short-lived protective efficacy. RTS,S has shown moderate to high initial vaccine efficacy that wanes quickly (*3*), suggesting that strategies to increase duration of responses would be valuable.

Memory B cells and long-lived antibody-secreting cells

The immunologic basis for durable immunity and how to generate sustained protection by vaccines remains poorly understood. For antibody responses, long-lasting protective immunity requires the induction of memory B cells that can mount a recall response upon reinfection and the generation of long-lived antibody-secreting cells (ASCs) that maintain circulating antibodies. Antibodies can also be maintained by the reactivation of memory B cells by polyclonal stimulation via microbial products such as lipopolysaccharide or unmethylated single-stranded DNA, which activates B cells via TLR4 and TLR9, or by bystander T cell help into short-lived ASCs that boost antibody production (*117*). Maximizing the initial induction of antibody functional activity, including optimal targeting of the most important epitopes, will be important, and the generation of multifunctional antibodies may help maintain efficacy over time. Studies show that recruitment of complement factors enables inhibitory or neutralizing activity at low antibody concentrations (*43, 50*), which may help maintain immunity as antibodies wane.

Antibody responses to malaria antigens are broadly regarded as relatively short-lived in the absence of ongoing exposure. For example, naturally acquired antibodies to merozoite antigens had reported mean half-lives of 0.6 to 8 years (depending on antigen) compared to extremely long-lived measles responses (457 years) in the same individuals (*118*). Models suggest that both short-lived and long-lived ASCs are responsible for circulating malaria antibody titers (*119*). The short-lived nature of acquired or vaccine-induced responses suggests suboptimal induction or maintenance of long-lived ASCs and memory B cells. This is in contrast to other vaccines that induce long-lived antibodies, for example, half-lives of 92 years after vaccinia vaccination, 11 years for tetanus, and 19 years of diphtheria, and many decades for measles (*120*). However, recent studies of naturally acquired immunity have shown that, despite

population-level short-lived responses, some individual children and adults do develop very long-lived antibodies and duration of responses differ between individuals, antigens, and response types (*121*), providing a proof of principle that durable immunity should be achievable by malaria vaccines.

Consistent with short duration of malaria antibodies, evidence from naturally acquired immunity suggests that the generation of memory B cell responses during malaria is suboptimal. In high-transmission settings, memory responses appear to require repeated *P. falciparum* infection and expand gradually over many years of exposure (*122*). However, like long-lived antibodies, robust memory B cell responses have been identified in some individuals from both high and low malaria transmission regions (*122, 123*). Encouragingly, memory B cells were induced by vaccination with RTS,S/AS01 in Gabonese children (*124*) and have been observed in whole parasite vaccine trials (*125*). Understanding the underlying basis for interindividual variation in induction and maintenance of long-lived protective responses is a key next step in identifying cellular events that have the potential to be manipulated to enhance vaccine-induced immunity. Recently, specific microRNA signatures that vary between individuals that predicted early control of parasite growth in CHMI, the induction of $CD4^+$ T cells and B cells, and the development of antibody responses (*126*) were identified.

Although further insights are needed to develop effective strategies, there are promising data on using adjuvants and TLR agonists to generate more durable immunity. A recent study in rhesus macaques using a Pfs25-synthetic particle vaccine demonstrated that the inclusion of TLR agonists R848 or CpG with the vaccine generated antibodies with significantly longer half-lives associated with induction of type I IFN polarization (*127*). With influenza, the use of AS03 adjuvant-induced memory B cells, $CD4^+$ T cells, and long-lived antibodies in humans (*128*), and the use of MF59 adjuvant in humans, has also been associated with long-lived antibodies and memory B cells (*129*). However, efficacy of the CSP-based vaccine R21 in mice was lower for MF59 than for other adjuvants (*115*). This difference from the influenza studies may possibly be due to a difference in responsiveness in human compared to animal models. Furthermore, combining multiple synergistic TLRs may increase plasma cell and antibody persistence (*130*).

Targeting T cell responses in memory development

T_{FH} cells are a key T cell subset required for the development of memory B cells and long-lived ASCs (*131*), and studies of influenza vaccination suggest that T_{FH} subsets influence the induction of protective antibodies and memory B cells (*69*). The importance of T_{FH} in immunity to *Plasmodium* has been suggested using mouse models (*132*). To date, a direct link between specific T_{FH} activation and protective antibody induction in malaria in humans has not been established.

Although research into improving T_{FH} activation during vaccination is in its infancy, there are indications that this is possible. For example, Clec9A antigen targeting of DCs promotes T_{FH} generation and antibody development in mice (*133*), including T_{FH} memory responses that reactivate after secondary challenge. In addition, the TLR9 agonist CpG increased T_{FH} activation in vitro (*134*). CpG has been tested in a phase 1 trial of malaria vaccine candidates AMA1-C1 and MSP1–42–C1 in malaria-naïve adults; the addition of CpG-7909 increased antibody and memory B cell development (*135*). However, large gaps in our understanding of the key underlying mechanisms of CpG activity remain. Recent studies found that, while CpG enhanced the magnitude of antibody responses to protein vaccines, it also blocked the ability of antigen-specific B cells to capture, process, and present antigen; to activate T cells; and thus failed to promote affinity maturation, including in the AMA1-C1 trial (*136*). To harness strategies to target T_{FH} in malaria vaccination, it is imperative to establish which specific subsets of T_{FH} are involved in protective malaria antibody induction and whether these responses are disrupted in malaria exposed populations.

Boosting of immunity by infection

In maintaining long-lived vaccine responses, it would be beneficial for recurrent parasite exposure to boost immunity and help maintain vaccine efficacy. An appeal of blood-stage vaccines is that boosting is likely to occur with repeated exposure because this is observed with naturally acquired immunity. With RTS,S, natural infections appear not to significantly boost vaccine responses and vaccine immunity wanes relatively quickly, reflecting the lower rate of acquisition of pre-erythrocytic immunity compared to blood-stage immunity (*43*). A possible explanation for this is that the number of sporozoites inoculated by mosquitoes is simply too low to provide the required antigenic stimulus necessary to boost memory B cells, although other factors may be important. Further research to understand how boosting of responses by infection could be achieved might be highly valuable toward maintaining pre-erythrocytic immunity. A similar lack of antibody boosting was observed in an AMA1 vaccine study where monkeys were administered three doses of recombinant *P. knowlesi* AMA1 vaccine, each followed by an infectious blood-stage challenge (*137*). Memory T cells were boosted by infection and evident for at least 200 days, highlighting differences across immune responses. Further investigation of possible cellular mechanisms underlying the lack of boosting (such as anergy of malaria-specific memory B cells or failure to induce memory T_{FH}) needs to be examined. In contrast, a trial in European adults vaccinated with merozoite antigens MSP1 and/or AMA1 (viral vectored) found that CHMI did lead to boosting of antibodies (*138*). In naturally acquired immunity, antibody concentration and rate of boosting by infection show variability depending on the antigen (*139*). Of the leading transmission-blocking candidates, Pfs230 and Pfs48/45 are targets of naturally acquired antibodies, whereas Pfs25 (or Pvs25) is not expressed in the human host; therefore, there is no opportunity for boosting with repeated exposure.

ASSESSING VACCINE EFFICACY IN CLINICAL TRIALS

Increasingly, vaccines are evaluated for efficacy using combinations of phase 2 field-based trials and CHMI trials. Phase 2 trials are ideal because they assess vaccine efficacy under conditions of natural exposure, with relevant antigen diversity and infecting dose conducted in representative target populations. However, disadvantages include costs and logistical challenges, larger sample size requirements, comorbidities that may affect immune responses and vaccine efficacy, and inability to control timing and level of exposure. Therefore, CHMI is a valuable additional strategy to assess candidates, formulations, and dosing before proceeding to field-based phase 2 trials and to identify correlates of protection. The CHMI model has been recently expanded to include assessment of transmission-blocking interventions (*140*). However, interpretation of early-phase challenge data requires careful consideration (*33*). Although CHMI has been

well established as a model for pre-erythrocytic vaccines, its utility for assessing blood-stage vaccines remains unclear; currently, there is a lack of data on blood-stage vaccine efficacy in CHMI versus phase 2 trials. Furthermore, the great majority of CHMI trials have been performed with only one *P. falciparum* strain, NF54.

An issue for consideration relates to differences in clinical thresholds for parasite density between healthy malaria-naïve adults in CHMI studies and children in malaria-endemic countries, the main target population. The symptomatic threshold for children in many endemic countries is typically much higher and can vary widely between 100 and 20,000 parasites/μl (*141*). In naïve volunteers, vaccine trials are halted at a low parasitemia for safety reasons (about 10 parasites/μl), typically ~10 days after inoculation and at about the same time that cellular immunity is only starting to have an effect. Thus, the lack of efficacy in CHMI should only be cautiously used as evidence that the vaccine will not work in an endemic country, particularly if the vaccine has induced a potent cellular immune response. Symptom threshold depends on numerous factors including antiparasite immunity (that will reduce the number of parasites), antitoxin immunity, and immune tolerance, whereby inflammatory immune responses that not only limit parasite growth but also cause immunopathology are dampened. Antibodies to parasites and parasite toxins or inflammatory mediators may decay at different rates after the last infection and may be differentially boosted. Maternal microchimerism, where children obtain maternal leukocytes and DNA during pregnancy or childbirth, can also alter sensitivity to malaria. Children with more maternal immune cells were more likely to acquire malaria in early childhood but were less likely to develop clinical symptoms, and maternal malaria was associated with increased microchimerism (*142*). Maternal malaria exposure also induces the differentiation of antigen-specific fetal effector T cells that are associated with protection from malaria in the first year of life (*143*). Recent investments in establishing CHMI capacity in malaria-endemic settings will help address these issues (*144*).

VACCINE IMPLEMENTATION

Future implementation of malaria vaccines will occur together with other control interventions, including insecticide-treated nets, indoor residual-insecticide spraying, intermittent preventive malaria treatment or chemoprophylaxis, and interventions such as mass drug administration or screen-and-treat campaigns. Hence, high vaccine efficacy may not be essential if vaccines are integrated with other interventions. RTS,S may be valuable in reducing seasonal malaria given its relatively high initial, but short-term, efficacy (*145*), although impacts on the broader EPI will need to be considered. A further consideration is that as transmission declines, disease burden shifts to older children and adults (*146*) because of a loss of acquired immunity. Similarly, the implementation of RTS,S or other vaccines in young children might lead to a shift in disease burden to older age groups. This may affect on the target population profile for vaccines over time.

Currently, there is a low coverage of existing malaria interventions in many countries (e.g., only half of the at-risk population sleep under a bed net) (*1*), and similarly, coverage of EPI vaccines is low in many malaria-endemic countries (especially measles given in the second year of life). This highlights challenges beyond vaccine development and the need for advances and innovations that would enhance vaccine implementation and integration of malaria vaccines into existing EPI schedules and malaria control interventions. Simplified and adaptable dosing schedules, or coformulation of malaria with other vaccines, would be an advantage. Greater durability of malaria vaccine efficacy would reduce demands on health care systems and provide flexibility for integration into EPI. Increased vaccine thermostability accommodates interruptions in the cold chain to allow implementation under the new WHO Controlled Temperature Chain, which can facilitate better coverage and reduce costs (*147*). Vaccines requiring more frequent schedules, prime-boost regimens, altered dosing regimens, or intravenous delivery all present substantial implementation barriers. Community perspectives on vaccine implementation and delivery are also important considerations. Research investments in these areas may be crucial for the long-term success of malaria vaccines, and considerations around future implementation early in vaccine development may be beneficial in the long term.

CONCLUSIONS AND FUTURE DIRECTIONS

The need for a highly effective and long-lasting malaria vaccine against *P. falciparum* and *P. vivax* remains strong. Recent demonstration of efficacy in endemic populations with the RTS,S vaccine and significant efficacy of several candidates in CHMI models have been important milestones in the quest for effective malaria vaccines. However, substantial challenges remain to advance beyond the generally modest efficacy demonstrated by existing vaccines tested in malaria-endemic areas and achieve the goal of a highly efficacious and durable vaccine, preferably for both *P. falciparum* and *P. vivax*. A deeper understanding of mechanisms and key targets of immunity is needed to underpin this, and research to reveal new strategies for the induction of a higher level of protective functional immunity. Achieving higher efficacy may require vaccines to induce multiple functional mechanisms and may require the inclusion of multiple antigens (of the same stage or across multiple stages), whole attenuated parasites, or innovative vaccine design to induce responses that better target specific protective epitopes compared to established strategies. A lack of correlates of protection to evaluate current vaccines and inform the development of new candidates is a constraint that hinders more expedient progress. Furthermore, most vaccines to date have induced protective immune responses of only short or modest duration, and new insights into how long-lasting immunity can be generated are urgently needed. Ideally, future vaccines will be low cost and affordable and administered in practical formulations and regimens suitable for mass vaccine campaigns and inclusion in childhood EPI. Although challenges remain, recent exciting progress and emerging knowledge promise great hope for the future.

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