

Vaccines to Accelerate Malaria Elimination and Eventual Eradication

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Remarkable progress has been made in coordinated malaria control efforts with substantial reductions in malaria-associated deaths and morbidity achieved through mass administration of drugs and vector control measures including distribution of long-lasting insecticide-impregnated bednets and indoor residual spraying. However, emerging resistance poses a significant threat to the sustainability of these interventions. In this light, the malaria research community has been charged with the development of a highly efficacious vaccine to complement existing malaria elimination measures. As the past 40 years of investment in this goal attests, this is no small feat. The malaria parasite is a highly complex organism, exquisitely adapted for survival under hostile conditions within human and mosquito hosts. Here we review current vaccine strategies to accelerate elimination and the potential for novel and innovative approaches to vaccine design through a better understanding of the host–parasite interaction.

Following the reinstatement in 2007 of global malaria eradication as a long-term goal, the malaria eradication research agenda initiative was conceived as a scientific consultative process between funding groups, researchers, and interest groups to identify key knowledge gaps and new tools required to move toward elimination and the eventual eradication of malaria. The crux of this strategy was enabling development of strategies and mechanisms to interrupt transmission of malaria, without which eradication would not be achievable. In 2011, a comprehensive R&D agenda was published (www.ploscollections.org/malERA2011; a new updated version will be available soon) in which

vaccine development was recognized as a key component, complementing other malaria interventions with the objective of interrupting transmission to bring about the eventual eradication of the parasite species responsible for causing malaria in humans. The MalERA agenda introduced the concept of vaccines to interrupt malaria (parasite) transmission (VIMT), which could potentially incorporate the classical transmission-blocking targets, the sexual/mosquito stages (transmission-blocking vaccine [TBV]); preerythrocytic vaccines that markedly reduce asexual- and transmission-stage prevalence rates; erythrocytic-stage vaccines that reduce asexual parasite and gameto-

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cyte densities to impact malaria transmission; and mosquito antigens to disrupt development in the vector. Although the main target product profile (TPP) of a VIMT is to interrupt transmission, an important additional benefit would be to provide protection against malaria symptoms and, ideally, to prevent epidemic spread following reintroduction after elimination.

A fundamental principle that underpins the current strategic agenda of VIMT development is that population bottlenecks (i.e., points in the life cycle where parasite numbers are low) constitute weak points where targeted approaches have a greater potential for success. As shown in Figure 1, there are two main opportunities for targeting parasite density bottlenecks: during the early exoerythrocytic phase with sporozoite injection and intrahepatocyte infection and development within the mosquito midgut following uptake of gametocytes. The fewer parasites infecting the host tissues, the reasoning goes, the greater the likelihood that induced immune mediators can prevent onward development. This is conceptually attractive as a vaccine strategy to prevent infection. Immunity in this vaccine-induced scenario differs from that of naturally acquired immunity to malaria, which occurs only after several exposures, largely to asexual targets, and acts to suppress parasite density and thereby prevent malaria symptoms. Another key advantage of a VIMT approach is a smaller population of parasites subjected to immune selection, reducing the probability of vaccine escape mutants. Naturally acquired immunity does not prevent infection or effect complete destruction of parasites in the host (Hoffman et al. 1987; Doolan et al. 2009). Thus, vaccine strategies focused on recapitulating naturally acquired immunity, via the targeting of asexual-stage antigens, are poorly aligned with the VIMT concept.

IMPORTANT CONSIDERATIONS FOR PRODUCT DEVELOPMENT OF A VACCINE TO ACCELERATE GLOBAL MALARIA ELIMINATION

What Does a Malaria Vaccine Need to Do?

The outputs from the MalERA processes, which informed the subsequent updates to the Malaria Vaccine Technology Roadmap (http://www.who.int/immunization/topics/malaria/vaccine_roadmap/TRM_update_nov13.pdf), have contributed to the establishment of clear community goals for development of a vaccine to interrupt malaria transmission. The 2013 updated Roadmap acknowledges the need for specific measures to tackle the burden of malaria caused by *Plasmodium vivax*, considered to be even more intractable than *Plasmodium falciparum* in some regions. The points below, generally presented in the context of *P. falciparum*, apply to *P. vivax* also.

In high-intensity transmission areas where malaria is not yet under control, a VIMT would synergize with existing or introduced control programs to move these regions toward elimination.

In areas where elimination has previously been achieved, such a vaccine would prevent reestablishment of transmission; and in the event of malaria resurgence (e.g., where previous control efforts have broken down [Cohen et al. 2012]), it would provide a safety net to prevent disease and death where naturally acquired immunity has waned (White 2014).

This issue is particularly relevant in the light of the spread of anopheline resistance to insecticides and the potential time bomb of resistance to artemisinin combination therapies (ACTs) crossing into Africa (Ashley et al. 2014; Mnzava et al. 2015).

Toward ensuring that such a vaccine meets requirements, the World Health Organiza-

Figure 1. Points of intervention of a malaria vaccine to accelerate toward elimination. (A) Within-host malaria parasite population dynamics showing bottlenecks post-mosquito injection and after uptake of the blood meal where the parasite is vulnerable to vaccine-induced immune mechanisms. (B) Vaccine-targetable processes within the *Plasmodium* life cycle. Areas susceptible to antibody-mediated mechanisms are shown in yellow and cell-mediated mechanisms in blue. This schematic does not account for the exoerythrocytic hypnozoite stage causing relapsing blood-stage *Plasmodium vivax* infections. RBC, Red blood cell; SPZ, sporozoite.

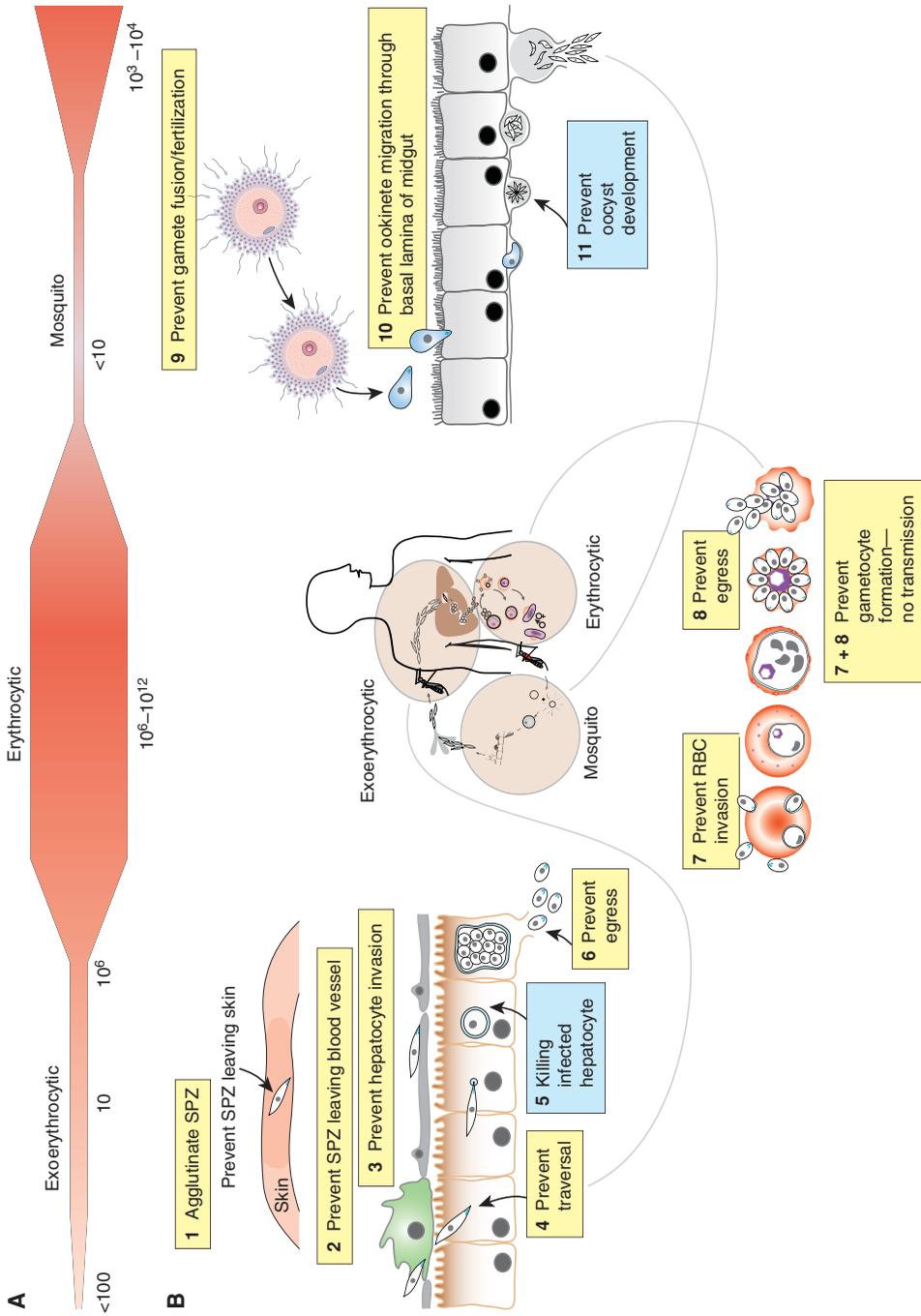


Figure 1. (Legend on facing page.)

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tion (WHO) published a set of preferred product characteristics (PPCs) that describe the parameters for the vaccine (apps.who.int/iris/bitstream/10665/149822/1/WHO_IVB_14.09_eng.pdf). These include indications, target groups, and the clinical data required to assess safety and efficacy.

A critical knowledge gap in the PPC guidelines currently is the target protective efficacy and coverage required to confer population immunity and impact malaria transmission, in particular transmission settings. Currently, mathematical modeling using clinical trial data is our only tool to advise on these parameters and to predict how vaccine efficacy will impact malaria transmission. The controlled human malaria infection (CHMI) model offers a practical way to obtain initial efficacy estimates of a candidate vaccine in naïve volunteers. Vaccine efficacy is generally measured by evaluating the proportion of vaccinated individuals who are protected following malaria exposure. In clinical field trials, a number of endpoints may be considered including *Plasmodium* infection, number of clinical episodes, time to infection, and the number of severe malaria cases (White et al. 2015). A critical measure of vaccine efficacy, and one that has proven particularly difficult to achieve to date, is duration of protection; models can provide insight into how waning immune responses will potentially impact on malaria transmission over time. For transmission-blocking vaccines, in which the definitive measure of efficacy will be via cluster-randomized studies (either pre- or postlicensure), pre-clinical and early clinical studies currently place a heavy reliance on the standard membrane feeding assay (SMFA) to measure transmission reducing and/or blocking activity. Although significant progress has been made in qualifying this assay, additional work is needed to better correlate outcomes with natural transmission measures (i.e., via direct skin feeding), as well as to translate “individual level” outcomes to anticipated impact on populations. Mathematical models of malaria transmission provide a rational approach to estimating the public health impact of these interventions (White et al. 2009); however, it is important that such

models are maximally informed by biological data, an example being the nonclinical population transmission model, which has yielded unexpected findings regarding the potential impact of relatively low efficacy interventions on sustained parasite transmission (Blagborough et al. 2013).

APPROACHES TO DEVELOPMENT OF VIMT

Attacking the Exoerythrocytic Cycle— Preventing Mosquito-to-Human Transmission

To enter the liver, intradermally deposited sporozoites must leave the skin by encountering and penetrating blood vessels (Sinnis and Zavala 2012). Mutant sporozoites with decreased migratory behavior and motility speed show dramatic reductions in invasion and infectivity, indicating that migration from the inoculation site poses a significant barrier and thus an ideal opportunity for intervention (Hopp et al. 2015). Available data indicate that fewer than 100 sporozoites result in hepatocytic infection following the bite of a mosquito (Fig. 1B-1) (Kebaier et al. 2009).

Following inoculation, sporozoites migrate around CD31⁺ endothelial junction cells before penetration of a blood vessel. Circumsporozoite protein (CSP) and thrombospondin-related adhesive protein (TRAP) have important roles in dermal migration (Fig. 1B-1,2) (Coppi et al. 2011; Ejjigiri et al. 2012). Sporozoites injected into the skin display a folded CSP conformation such that the amino-terminal region masks the carboxy-terminal thrombospondin repeat (TSR) domain. Although a number of sporozoites enter lymphatic vessels and do not contribute to hepatic invasion (Amino et al. 2006), these are crucial in priming CD8⁺ T-cell immunity that targets intrahepatocytic infection (Fig. 1B-5) (Radtke et al. 2015). How sporozoites recognize liver proximity and by what mechanisms they exit the blood vessel and initiate liver cell entry are unknown, but an understanding of these processes would provide insights for targeted vaccine development (Fig. 1B-3).

On reaching the liver, the amino-terminal region of CSP is proteolytically cleaved, expos-

ing the TSR that is crucial for hepatocyte adhesion and invasion (Coppi et al. 2005, 2011). Antibody-mediated prevention of this processing event inhibits sporozoite invasion in vitro (Espinosa et al. 2015), indicating that incorporating amino-terminal CSP targets may improve the efficacy of a CSP-based vaccine. Because the proteolytic cleavage site (region I) appears to be genetically conserved across species, it represents a promising target for induction of cross-species protection.

The liver vasculature, or sinusoids, is comprised of two cell types, the endothelial and macrophage-like Kupffer cells (Widmann et al. 1972). Kupffer cells are the main route of entry for sporozoites into the liver parenchyma (Meis et al. 1983; Frevert et al. 2005; Baer et al. 2007), although endothelial cells have also been observed as a route of sporozoite entry into the liver (Fig. 1B-3,4) (Tavares et al. 2013). Sporozoite traversal occurs via active penetration rather than phagocytosis (Vanderberg and Stewart 1990; Frevert et al. 2005) and CD68 is a receptor for Kupffer cell invasion, although the sporozoite ligand is not yet known (Cha et al. 2015). This would be a prime target for vaccination. How the sporozoite escapes triggering the respiratory burst is not completely understood, but involves CSP binding (Usynin et al. 2007); thus, antibodies bound to CSP may act to reverse this respiratory burst inhibition or provide an opsonization signal for phagocytic activity by Kupffer cells.

Once the process of traversal is complete and a suitable hepatocyte is identified, the process of active invasion occurs, leading to the establishment of the parasite inside a parasitophorous vacuole (PV) (Sibley 2011). The molecular determinants of this process are poorly understood but involve interactions between sporozoites and specific surface receptors on hepatocytes. These cells are recognized and bound by the amino-terminal region of CSP, mediating a signal for hepatocyte invasion (Coppi et al. 2007). Hepatocyte membrane-bound CD81 is one important receptor for sporozoites (Silvie et al. 2003), and EphA2, which associates with sporozoite proteins P52 and P36, is another (Kaushansky et al. 2015). Intra-

hepatocytic replication commences inside the PV. This provides a protective niche and prevents fusion with endosomal compartments. Once a liver schizont completes development, merozoites are released, initiating the self-replicating, pathogenic blood-stage cycle of malaria.

A fully effective vaccine that prevents sporozoite invasion of hepatocytes would also prevent emergence of blood-stage parasites, including gametocytes, and thus parasite transmission (inducing so-called sterile immunity).

Proof-of-principle evidence that sterile protection is achievable by vaccination comes from studies of irradiated sporozoites in humans and animal models (Nussenzweig et al. 1967; Clyde et al. 1973; McCarthy and Clyde 1977; Rieckmann et al. 1979). Complete protection against a sporozoite challenge can be induced when subjects are immunized with adequate numbers of attenuated parasites. Radiation-attenuated sporozoites undergo random DNA damage and arrest early in intrahepatocytic development, before DNA replication. What is not yet clear is the exact gene expression attenuation profile required to inactivate parasite development while still inducing protective immunity; nor is it clear what immune profile confers protection in protected individuals, although associations have been shown with CD8⁺ T-cell responses targeting liver-stage antigens (Epstein et al. 2011; Seder et al. 2013). Answers to these questions will provide direct routes to targeted vaccine development, including development of genetically attenuated sporozoite vaccines, which are potentially safer in terms of quality control than irradiated vaccines, because identical clonal parasite lines are used in which the exact gene attenuation profile is known (Vaughan et al. 2010). These approaches benefit, at least in early-stage development, from the absence of a requirement to define specific protective antigens (a critical requirement for subunit vaccine development) and have the potential to enable identification of immune correlates of protection that could inform subunit vaccine development. The most advanced whole-parasite vaccine effort involves radiation-attenuated *P. falciparum* sporozoites, administered by the intravenous route. This ap-

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proach, being developed by Sanaria (Rockville, MD), protected six out of six volunteers in the highest dose group from infection in CHMI studies (Richie et al. 2015). Studies are ongoing to replicate these initial findings in larger numbers of volunteers, and to generate evidence for sustained protection (via delayed challenge) and cross-strain protection (via heterologous challenge). Initial results from a field study in Mali have revealed a more modest level of efficacy (approximately 50% protection over 6 months of follow-up) (ECTMIH Special Issue 2015, <http://www.ectmihbasel2015.ch/ectmih2015/home>). Further, in view of the manufacturing substrate (sterile mosquitoes) reservations remain as to the feasibility of producing adequate material for global mass vaccination campaigns, as well as lack of alignment with current WHO PPCs for next-generation malaria vaccines, specifically with respect to need for intravenous delivery and a liquid nitrogen cold chain (apps.who.int/iris/bitstream/10665/149822/1/WHO_IVB_14.09_eng.pdf).

It has been postulated that the former issue, the numbers of irradiated sporozoites required, may be circumvented by adopting a similar strategy, known as ChemoProphylaxis with Sporozoites (CPS), where complete protection is induced by a fraction of the dose required in the *Plasmodium falciparum* sporozoite (PfSPZ) vaccine (Roestenberg et al. 2009). However, recent enthusiasm for the potential impact of this approach has been tempered by the report of disappointing levels of heterologous protection (Schats et al. 2015); further, challenges with ensuring safe and effective delivery of such a vaccine approach remain to be resolved.

Late liver-stage parasites show an overlapping transcriptomic profile with blood-stage parasites (Tarun et al. 2008) suggesting at least some of these antigens are expressed in both stages. Indeed, immunization with a late-arresting genetically attenuated parasite (GAP) did provide some protection against a low-dose blood-stage challenge, by a cell-mediated mechanism; however, in a CPS human challenge model, no protection was observed against direct blood challenge (Bijker et al. 2013). It has been determined that generation of preery-

throcytic immunity is dependent on sporozoite numbers (Bijker et al. 2014; Nahrendorf et al. 2015). This is consistent with the assertion that naturally acquired preerythrocytic immunity is absent in malaria-endemic situations (Tran et al. 2014). Protection against challenge in a rodent study model was only apparent after several cycles of replication in the bloodstream and indicates that lack of protection against blood-stage challenge in human volunteers may have resulted from the necessarily early treatment with chloroquine (CQ), before the onset of symptoms. Attenuation by other means, either chemical (Good et al. 2013) or genetic (Aly et al. 2011), could potentially provide protection against homologous and heterologous blood-stage challenge. Complete sterilizing immunity against sporozoite challenge in a late-arresting GAP liver infection model is conferred by only two, rather than three, immunizations in other GAP/RAS models, suggesting that late schizont/merozoite antigen expression may be important. This is supported by evidence that MSP1 can induce a multistage immune response with partial protection against liver stages (Draper et al. 2009).

The malaria vaccine candidate most advanced in development is RTS,S, comprising Hepatitis B surface antigen (HBsAg) and an HBsAg-circumsporozoite (CS) fusion protein, which are produced in yeast and form a particulate structure. It is formulated with the proprietary AS01 adjuvant system of GlaxoSmithKline (GSK). Results after a year of follow-up in a phase III efficacy (clinical malaria endpoint) and safety trial, involving more than 15,000 infants and young children, at 11 sites in seven African countries, showed that three doses of RTS,S reduced clinical malaria by approximately half in children 5–17 months of age at first vaccination (Agnandji et al. 2011). The final study results analyzed vaccine efficacy (VE), immunogenicity, safety, and impact of RTS,S/AS01 over a median of 38 and 48 months of follow-up (postdose 1) in infants and young children, respectively, including the effect of a fourth dose of vaccine (Greenwood 2015). These final results showed that vaccination with the three-dose primary series reduced clinical malaria cases by 28% (95% CI: 23.3; 32.9) in

young children and 18% (95% CI: 11.7; 24.4) in infants to the end of the study. Adding a fourth dose of RTS,S, administered 18 months after the primary series, reduced the number of cases of clinical malaria in young children by 36% (95% CI: 31.8; 40.5) and in infants by 26% (95% CI: 19.9; 31.5) over the entire study period. Therefore, although the VE waned over time, the fourth dose contributed to enhancing VE, but not to the level observed following the first three doses. These results were achieved within the context of existing malaria interventions, such as insecticide-treated nets (ITNs), which were used by approximately 80% of the trial participants during the follow-up period.

In June 2014, GSK submitted an application for a scientific opinion on the RTS,S/AS01 candidate vaccine to the European Medicines Agency (EMA) under its Article 58 procedure. The EMA's Committee for Medicinal Products for Human Use (CHMP) evaluated data on the quality, safety, and efficacy of the RTS,S/AS01 vaccine candidate. In July 2015, CHMP adopted a positive scientific opinion for RTS,S/AS01, stating that the quality of the vaccine and the benefit-risk balance are considered favorable from a regulatory perspective. However, the EMA also requested that additional information needs be addressed in future studies, specifically with respect to (1) the timing of the fourth dose and evaluation of the safety and efficacy of an earlier fourth dose; (2) the efficacy and safety of multiple yearly doses and whether the vaccine predisposes to some degree of hyporesponsiveness to sequential doses; and (3) the potential utility of a delayed and fractionated third dose schedule in the target age group. Subsequent to the Article 58 process (WHO issued formal recommendations in January 2016 calling for large-scale pilot implementations of RTS,S; www.who.int/immunization/research/development/malaria_vaccine_qa/en).

The recommendations include that the pilot implementations use the four-dose schedule of the RTS,S/AS01 vaccine in three to five distinct epidemiological settings in sub-Saharan Africa, at the subnational level, covering moderate-to-high transmission settings, with three doses administered to children between 5 and 9 months

of age, followed by a fourth dose 15–18 months later. Further recommendations for the pilot implementations are outlined in the WHO position paper.

Concurrent with the activities associated with the conduct and regulatory submission of the phase 3 RTS,S/AS01 clinical trial, PATH's (Program for Appropriate Technology in Health) malaria vaccine initiative (MVI) and GSK have continued to investigate opportunities to increase the efficacy and duration of protection against malaria infection toward development of a vaccine that could contribute to malaria elimination and eradication. An important milestone was achieved in 2014–2015, when an alternative regimen of RTS,S/AS01—in which the third dose is delayed by 6 months and fractionated to one-fifth of the standard dose (thus the term “FxRTS,S”)—achieved 87% protection (95% CI: 67–95), compared to 63% (95% CI: 20–80) for the standard full-dose regimen, in a CHMI study. The fractional regimen increased antibody somatic hypermutation and avidity. Rechallenge showed waning efficacy in both groups, but fractional dose boosting maintained high protection (manuscript in preparation). These results were remarkably similar to findings reported in 1997, where six of seven volunteers were protected with a similar regimen of RTS,S/AS02 (Stoute et al. 1997); this regimen was not pursued at that time, as the results were generally viewed as being a “chance” finding in view of later studies (where the fractional booster dose was not implemented), and the nonalignment with the accepted expanded program on immunization (EPI) schedules, for which the vaccine was intended. The GSK/PATH partnership are proceeding to a phase 2b field study in young African children (aged 5 to 17 months at first vaccination) to evaluate the potential of FxRTS,S to prevent naturally acquired *P. falciparum* infection.

PREVENTION OF HUMAN–MOSQUITO TRANSMISSION—ATTACKING THE ERYTHROCYTIC STAGES

Early malaria vaccine development efforts focused on induction of an immune response that would clear or restrict fulminating parasite-

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mia, thus preventing severe disease and death. The rationale for this approach came from resolution of malaria symptoms following clinical interventions involving passive transfer of hyperimmune adult sera to children experiencing severe malaria symptoms with high parasitemia (Cohen et al. 1961; Sabchauer et al. 1991). The prospective targets for conferring such immunity were antigens expressed in the blood stages, many of which were correlated with immune protection in endemic regions or were identified by screening expression libraries with hyperimmune sera. Thus, there was a clear correspondence between induction of vaccine-induced protection and naturally acquired immunity to malaria. Despite a pressing medical need for a vaccine to prevent clinical malaria, as well as a strong rationale for vaccine-induced immunity against blood stages to provide a safety net in regions of waning naturally acquired immunity because of reductions in parasite transmission achieved by other measures, blood-stage vaccine research has been deprioritized in favor of targeting preerythrocytic- and mosquito-stage parasites. Reasons for deprioritizing blood-stage vaccines in an elimination agenda include the bottleneck phenomenon (Fig. 1): the perception that large numbers of circulating parasites, and genetic diversity of many, but not all potential asexual blood-stage target antigens, present an insurmountable hurdle to the vaccine-induced immunity. The disappointing clinical efficacy of AMA1- and MSP1-based vaccines have no doubt contributed to the idea that blood-stage parasitemia is intractable to control by vaccination (Spring et al. 2009; Sheehy et al. 2012; Payne et al. 2016). These unsuccessful clinical trials have highlighted the importance of judicious antigen choice, with conserved antigens preferable to polymorphic ones and prioritization of antigens capable of evoking protective responses at relatively low antibody concentrations.

These trials highlighted the benefit of early clinical testing of prospective approaches in CHMI models, toward determining whether induced responses reduce parasite replication in vivo, and CHMI is increasingly serving as a stage gate before endemic field testing (Duncan et al. 2011; Duncan and Draper 2012).

It still remains possible that a highly effective blood-stage immune response could form an integral part of a VIMT strategy if it successfully reduced death, disease, and transmission by limiting the asexual parasite density below a threshold required for infectious gametocyte production. How these factors are related is not well understood, however, and more research is required to address this particular knowledge gap.

The leading blood-stage vaccine candidate today is PfRh5, a conserved merozoite protein essential to erythrocyte invasion (Fig. 1B-7) (Baum et al. 2009; Douglas et al. 2011, 2015; Chen et al. 2014; Wright et al. 2014). Other merozoite protein clinical candidates remaining in the vaccine pipeline from the pre-eradication era are SERA5, MSP3, MSP1, GLURP, AMA1, and EBA-175 (Birkett 2015). Promising early clinical trial data support further investigation into MSP3 (Sirima et al. 2011) and SERA5 (Palapac et al. 2013) as possible components in a next-generation malaria vaccine. Another antigen showing preclinical promise is schizont egress antigen-1 (SEA-1). This protein plays an essential role during schizont rupture during the blood stage (Fig. 1B-8) and carriage of antibodies to this protein was associated with reduced incidence of severe malaria (Raj et al. 2014). Whether antibodies to SEA-1 can also block liver schizont egress (Fig. 1B-6) is not known, but an essential process that could be blocked in multiple stages with a single vaccine antigen should be considered as a high priority for further investigation.

Antigens present on the surface of the parasitized erythrocyte membrane are associated with natural immunity to malaria; however, their high diversity presents a major roadblock for vaccine design. A vaccine aimed to prevent pregnancy-associated malaria, based on the relatively conserved PfEMP1 protein VAR2CSA, is close to clinical transition (Birkett 2015).

During a blood-stage infection, a proportion of parasites will divert to a sexual course of development, dependent on the uptake of mature gametocytes by a feeding mosquito. Conversion to gametocyte development is a poorly understood process (see Meibalan and Marti

2016 for details) that is constitutive in *Plasmodium*, but is also inducible by certain environmental conditions, indicating that parasites sense changes in their environment and respond to these cues. In *P. falciparum*, intracellular gametocytes develop through five distinct stages, and sequester in the bone marrow and spleen for 9–12 days until mature forms emerge into the peripheral circulation and are transmissible. Many sexual stage-specific antigens are expressed in the developing gametocyte, but do not play a role in nutrient acquisition or cytoadherence, and thus are not revealed to the immune system by presentation on the erythrocyte surface.

Until recently, it was widely held that circulating gametocytes were immunologically silent. The proteins exposed on the surface of gametocyte-infected erythrocytes are potentially involved in cytoadherence, conferring the ability of maturing stages to sequester until maturity, are poorly characterized but may include variant proteins such as STEVOR (McRobert et al. 2004), RIFIN (Sharp et al. 2006) or others yet to be identified (Saeed et al. 2008). This as-yet-unexplored area could present new opportunities for gametocyte-specific immune targeting to reduce transmission and move toward malaria elimination.

PREVENTION OF HUMAN–MOSQUITO TRANSMISSION—ATTACKING THE MOSQUITO STAGES

Once inside the midgut of the mosquito, the changed environment signals the gametocytes to break down the erythrocyte membrane and

emerge as male and female gametes that can undergo fertilization. At this point, well-characterized antigens (clinical vaccine candidates Pfs230 and Pfs48/45) are exposed and are targetable by antibodies and complement (Table 1) (Sauerwein and Bousema 2015).

Mosquito midgut invasion by *Plasmodium* ookinetes is considered a promising target for transmission-blocking intervention, as parasite numbers undergo a major bottleneck at this stage (Sinden 2010). After the mosquito ingests an infected blood meal, male and female gametes fuse in the midgut lumen, giving rise to zygotes that differentiate into motile ookinetes.

After crossing the peritrophic matrix aided by chitinase secretion (Tsai et al. 2001), the ookinete establishes specific molecular interactions with the midgut epithelial cells, followed by their invasion and traversal. Several proteins from the ookinete, including the most advanced TBV candidate Pfs25 and the mosquito aminopeptidase 1 (AnAPN1), may be involved in this process (see Bousema and Drakeley 2016 for more details). AnAPN1 presents a conceptually attractive candidate because it potentially targets multiple *Plasmodium–Anopheles* species combinations, although preclinical studies have yet to confirm this. The only molecular interactions between the ookinete and the midgut characterized thus far are the in vitro interaction between parasite Pvs25 and mosquito calreticulin (Rodriguez Mdel et al. 2007) and between the ookinete enolase and the mosquito midgut enolase-binding protein (Vega-Rodriguez et al. 2014).

Table 1. Malaria vaccine candidates in clinical trials

Exoerythrocytic antigens	Erythrocytic-stage antigens	Transmission blocking	Multistage
RTS,S (CSP)	PfAMA1 (3)	Pfs25 + Pfs230-EPA	PfCSP+AMA1
PfME-TRAP	PfAMA1+MSP1		PfTRAP+MSP1
PfSPZ (RAS, CPS)	PfGLURP+MSP3		PfTRAP+RH5+Pfs25
PfCSP (3)	EBA-175		
PfCelTOS	PfRH5		
PvCSP	SERA5		
PfGAP (2)	PvDBP		

For up-to-date information, see the WHO rainbow tables at: www.who.int/immunization/research/development/Rainbow_tables/en.

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The classical TBV candidates are antigens expressed on the surface membrane of gamete, zygote, and ookinete forms. The best characterized of these, Pfs25, is the lead TBV in clinical development. However, challenges in the induction of high titer antibody responses have been recently reported, with poor immunoreactivity in humans with this antigen, despite good pre-clinical results in nonhuman primates. For an in-depth recent review of the TBV candidate development status see Nikolaeva et al. (2015).

Two of the main challenges in TBV development have been: (1) producing immunogens with three-dimensional structures that mimic their native counterparts, and (2) inducing in humans the levels of functional antibodies observed in preclinical models. Although progress has been made in qualifying the SMFA (Miura et al. 2013), there remains a need to better understand the correlation between the readout of assays used to report the impact of TBVs and the longitudinal impact on malaria transmission (Blagborough et al. 2013; Kapulu et al. 2015). A rapid pipeline of proof-of-principle human trials for testing lead candidates would improve prioritization of new TBV candidates (Sauerwein and Bousema 2015). Target identification efforts that leverage available sera with high levels of naturally induced transmission-blocking activity have recently been initiated.

INNOVATION FOR DISCOVERY OF NEW TARGETS AND BIOMARKERS

Publication of the *P. falciparum* (Gardner et al. 2002) and *P. vivax* (Carlton et al. 2008) genomes revealed many novel potential candidate antigens. Transcriptome (Le Roch et al. 2003; Silvestrini et al. 2005; Xu et al. 2005; Young et al. 2005) and proteomic (Lasonder et al. 2002; Hall et al. 2005; Khan et al. 2005; Lal et al. 2009) studies have since confirmed expression of hundreds of *Plasmodium* proteins. Potentially, all of these could be investigated for their suitability as vaccine antigens and their roles in parasite development. However, only a few have been the subject of preclinical studies. Advances in other postgenomic areas such as metabolomics,

lipidomics, and functional genomics, particularly with CRISPR/Cas9 technology to expedite large screening projects, are also poised to yield exciting leads in vaccine R&D.

Conventional screening approaches used for antigen identification to date have included expression cloning of putative candidates followed by immunoreactivity testing of plasma by ELISA or western blot; elution and mass spectrometry sequencing of MHC-bound peptides; and in vitro testing of pools of overlapping peptides and reverse immunogenetics (Doolan et al. 2008). However, these methods are not applicable to high-throughput analysis of genomic data. Screening of protein microarray (Doolan et al. 2008; Felgner et al. 2013) or expression libraries (Richards et al. 2013; Arumugam et al. 2014; Osier et al. 2014) can be a useful alternative for identifying immunodominant antigens and immunoreactivity profiles from donors with defined malaria immune status. Antibody profiles from naturally immune individuals and those from CPS- or radiation-attenuated immunized, protected individuals have distinctly different profiles (Felgner et al. 2013), with a strong blood-stage bias in protective immunity from natural infection compared with largely preerythrocytic or mixed-stage targets via CPS vaccination, which is perhaps unsurprising given that CQ treatment is highly effective in preventing establishment of a blood-stage infection.

A key knowledge gap is what constitutes a protective immune response. This is used to inform which particular antigens or epitopes are likely to comprise the best targets, whether adjuvants will be required to optimize the induction of immune effectors, and which platforms may be best suited for durability and longevity of the protective response. Rational design of malaria vaccines can be facilitated by progress in the understanding of the host–parasite relationship, particularly, mechanisms of host protective immunity and molecular mechanisms of pathogen–host interactions, particularly those that are essential to parasites' survival and reproduction, and also those used to evade host immunity. To investigate protective immunity a combination of systems biology, -omics

technologies, interrogation of the B-cell repertoire, cell-based assays, and epidemiological studies are all relevant.

The relative importance of different immune responses in endemic settings is difficult to gauge, inconsistent between studies and varies with seasonality and parasite density. Despite extensive use as surrogate markers of immunity, neither antibody levels nor interferon γ (IFN- γ) responses correlate consistently with protection between studies (Dunachie et al. 2015). Of particular note is the failure of the parasite growth-inhibition assay (GIA) to predict clinical outcomes from trials of blood-stage vaccines to date.

The availability of highly characterized parasites for challenge via CHMI offers significant advantages over the study of natural infections for gaining insight into protective mechanisms of antimalarial immunity following vaccination or exposure (reviewed in Sauerwein et al. 2011). In a CHMI study, participants are infected with parasites delivered via three alternative routes: with sporozoites via mosquito bite (Chulay et al. 1986; Roestenberg et al. 2012); via needle-based inoculation of cryopreserved sporozoites (Epstein 2013); or via thawed cryopreserved, infected red blood cells (Lawrence et al. 2000; Pombo et al. 2002; Duncan et al. 2011). CHMI by mosquito bite challenge has been the most widely adopted, and provides a cost-effective, highly reproducible assessment of vaccine candidates for their efficacy against infection. Two groups have reported successful challenge with *P. vivax*-infected mosquitoes opening up the potential for much-needed research on *P. vivax* vaccines (Herrera et al. 2009; Bennett et al. 2016).

Vaccine approaches designed to interrupt transmission of *P. vivax* parasites must account for two unique features that are distinct from *P. falciparum* parasites: (1) the relapse potential of liver-resident hypnozoites; and (2) accelerated transmissibility associated with direct emergence of gametocytes from the liver at the initiation of the asexual blood-stage infection. A highly efficacious preerythrocytic vaccine that prevents initial infections, and thereby preventing relapsing hypnozoites, has significant po-

tential in accelerating *P. vivax* elimination. Although relatively few *P. vivax* vaccine approaches have been tested in human clinical trials, a chimeric PvCSP protein (VMP001), developed by Walter Reed Army Institute of Research (WRAIR) and incorporating a truncated repeat region containing sequences from both the VK210 (type 1) and the VK247 (type 2) parasites has been evaluated in a single CHMI study. Although all volunteers immunized with VMP001/AS01 progressed to blood-stage parasitemia following challenge by infected mosquitoes, a significant delay in the median prepatency period was noted as compared to the infectivity controls (Bennett et al. 2016). These results suggest that induced immune responses partially blocked hepatocyte invasion by sporozoites and/or emergence of merozoites from infected hepatocytes. The development and testing of a particulate CS-based immunogen, and application of the delayed fractional booster dosing regimen, successfully applied to RTS,S/AS01, would appear to be logical next steps.

SPECIAL CONSIDERATIONS FOR A TBV

Classical TBVs, also known as SSM-VIMT (for sexual, sporogonic, and/or mosquito-stage vaccines interrupting malaria [parasite] transmission), target the mosquito stages of the life cycle by inducing antibodies within the human host that prevent onward transmission and, therefore, provide no direct and immediate clinical benefit to the vaccine recipient. Delayed individual benefit would be realized at the population level, when transmission is decreased or ceased. Despite the absence of direct and immediate benefit to the vaccine recipient, the United States Food and Drug Administration (FDA) has asserted that there is no legal bar to prevent a vaccine such as an SSM-TBV from being considered for licensure in the context of their review process. This is consistent with the continued implementation of pertussis vaccine, which from a public health standpoint primarily benefits the unborn children of pregnant women. Further, tetanus and influenza vaccines (along with pertussis) are recommended during preg-

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nancy, and vaccine development efforts for respiratory syncytial virus (RSV) and group B streptococcus (GBS) are increasingly targeting maternal immunization as an approach to benefit newborns (Lindsey et al. 2013).

Another key consideration is how to monitor the efficacy and secure licensure of such a vaccine via clinical trials. Two approaches have been proposed: (1) a large-scale, phase 3 efficacy trial, which, in the case of an SSM-VIMT, has been proposed by regulators to be a cluster-randomized trial (CRT) to show vaccine impact on incidence of infection in the community; and (2) an accelerated approval pathway in which the vaccine would receive approval for use based on an analytically and biologically, but not clinically, validated surrogate of efficacy (which does not yet exist), with impact on transmission confirmed during postapproval studies (Nunes et al. 2014; Delrieu et al. 2015). The accelerated approval strategy would likely involve feeding mosquitoes directly on malaria-exposed trial participants or via drawn blood in membrane feeders. However, there are likely to be significant differences in transmission between humans and mosquitoes (e.g., age of volunteer, immune status, parasite and mosquito genotypes) (Smith et al. 2010; Tusting et al. 2014; Reiner et al. 2015); thus, trial size, as well as design, will be major considerations in ensuring statistical confidence in the results.

Further challenges are how to predict the public health impact from reducing transmission, and what criteria to apply regarding the minimal efficacy required to ensure that the significant financial and logistical investment required of such a vaccine is warranted.

TARGET POPULATION FOR A VACCINE TO ELIMINATE MALARIA

All individuals with the potential to become infected, including those who are asymptomatic and whose parasitemia is below detectable levels, should be considered capable of transmitting malaria parasites; therefore, everyone in the area of transmission would likely need to be immunized with a VIMT. This contrasts with

vaccine approaches intended to protect only those at greatest risk from the severe consequences of malaria, primarily young African children, who are targeted via the EPI schedule. Distinct implementation strategies, and other key considerations that need to be considered in development of these two classes of vaccine, have been the focus of increased recent attention (Nunes et al. 2014; Birkett 2015).

CONCLUDING REMARKS

The first 15 years of the 21st century have witnessed remarkable reductions in mortality attributable to malaria, in alignment with the coordinated implementation of a range of interventions. Approximately six million lives, mostly of young African children, have been spared during this period (www.who.int/malaria/media/world-malaria-report-2015/en). Emerging threats to the effectiveness of these interventions highlights the need for new and innovative approaches. The potential for a vaccine to contribute to maintaining the gains and potentially contributing to further reductions in disease and death has become closer to realization over the past 2 years with RTS,S/AS01, the most advanced vaccine candidate in development globally, completing phase 3 testing and receiving a positive opinion from the EMA. The future impact of RTS,S/AS01 will be defined by its performance in pilot implementations recommended by WHO to generate the supplemental evidence needed to support possible wide-scale implementation.

In the face of these advancements, the vaccine development community has been challenged to apply the proven attributes of this powerful intervention to contribute to reduced transmission, toward accelerating elimination and eventual eradication of the parasites responsible for causing malaria in humans. This has led to an increased focus on research to better understand the biological process associated with parasite invasion, particularly at the interface of the two obligate hosts, humans and female anopheline mosquitoes, critical for maintaining parasite transmission. Further un-

derstanding these invasion mechanisms could reveal potent new vaccine targets.

Vaccine development efforts, utilizing an array of subunit and whole parasite vaccine approaches, are increasingly focused on infection and transmission endpoints. Short-term protection levels (from infection) approaching 100% have recently been reported for two distinct vaccine approaches, RTS,S/AS01 and PfSPZ, in CHMI studies, resulting in significant optimism. For these leading approaches, efforts are underway to improve the durability of protective responses and ease implementation challenges. As these approaches transition to endemic field testing for assessment under conditions of natural transmission, clear “lines of sight” for development, regulatory approval, implementation, and financing will become increasingly important.

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