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Malaria Transmission-Blocking Vaccines: Present Status and Future Perspectives

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Abstract

Transmission-blocking vaccines (TBVs) utilize *Plasmodium* sexual stage proteins to induce antibodies that prevent parasites from infecting blood-fed mosquitoes. This type of vaccine, which can be considered a "vaccine of solidarity," reduces *Plasmodium* infections within communities without conferring direct protective immunity to the vaccine recipients. The leading TBV candidates have advanced to field clinical trials, where vaccine-induced antibody function has been demonstrated in mosquito-feeding assays. However, the duration of functional antibody responses has been short-lived; hence current development has focused on improved adjuvant and vaccine delivery systems to generate long-lasting immune responses. For the future implementation of TBVs, community perceptions and understandings should be considered, and education should be provided on the concept and its value. Implementation will need to be undertaken in harmony with current malaria control policies.

Keywords: transmission-blocking vaccine, malaria, mosquito-feeding assays, elimination, eradication

1. Introduction

Malaria elimination and eradication have received renewed attention as the best long-term solution to this ancient scourge. However, existing tools that have been programmatically implemented have been insufficient to achieve elimination in areas of Africa, as well as at a global scale. Thus, new products are needed to pursue elimination, and efficacious vaccines are generally conceded to be ideal population-based interventions to support disease eradication. In this chapter, we describe the rationale and status of transmission-blocking vaccines

(TBV), which will contribute to malaria elimination and eradication. We place TBV in the context of overall malaria vaccine development and highlight the role and challenges of TBV field trials, which are needed to confirm activity and guide implementation.

2. Current malaria burden and need for elimination/eradication

Malaria is the most important parasitic disease and is endemic across the globe, most importantly sub-Saharan Africa, South and Southeast Asia, Papua New Guinea, and South America. Its burden remains unacceptably high, especially in sub-Saharan Africa, despite the significant gains with the use of current tools including vector control, diagnostics, chemoprevention and treatment. In 2016, 216 million malaria cases and 445,000 deaths were recorded worldwide mostly caused by *Plasmodium falciparum,* 90% of which occurred in sub-Saharan Africa [1]. Since artemisinins constitute the core component of the current malaria treatments, the recent emergence of artemisinin resistance in Southeast Asia [2–4] has become a serious obstacle for the malaria elimination agenda. Although the current phenotypes of artemisinin resistance are limited to slow parasite clearance and parasite recrudescence, their impact in malaria-endemic areas could result in a considerable increase of malaria cases, deaths, and economic costs according to predictive models [5, 6]. Indeed, the probable spread of artemisinin resistance to sub-Saharan Africa, where the burden of malaria is the highest, could jeopardize the lives of millions of children. Furthermore, the spread of insecticide and mosquito behavioral resistance compromises malaria control via the failure of vector control interventions such as indoor residual spraying (IRS) and insecticide-treated nets (ITN). Given that current strategies will eventually fail, new tools are urgently needed for malaria control and treatment. To overcome these constraints, transmission-blocking vaccines (TBVs) offer a new approach by targeting developing parasites in the mosquito host (a bottleneck in the malaria parasite lifecycle) and thereby contributing to malaria elimination and potentially eradication.

3. Need for malaria vaccine strategy

Vaccines are powerful tools that could accelerate malaria elimination efforts. Historically, vaccine-based strategies have contributed to the successful eradication of infectious diseases in humans and animals, including smallpox and rinderpest [7, 8]. Poliomyelitis is now close to eradication through routine Expanded Programme on Immunization (EPI) and massive immunization campaigns in some areas. Vaccination is a safe and cost-effective strategy that is easily implemented in large populations to reduce or even eliminate disease morbidity and mortality. Vaccine-induced immune responses protect individuals against infection or disease and can also stop transmission of the causative agent. With high coverage, vaccines protect not only recipients but also non-immunized individuals within the population through the effect of herd immunity. Malaria vaccines, even those with modest efficacy, such as the RTS,S product (see below in "Current status of malaria vaccine research"), are expected to avert millions of clinical malaria cases and thousands of severe malaria cases, hospitalizations, and
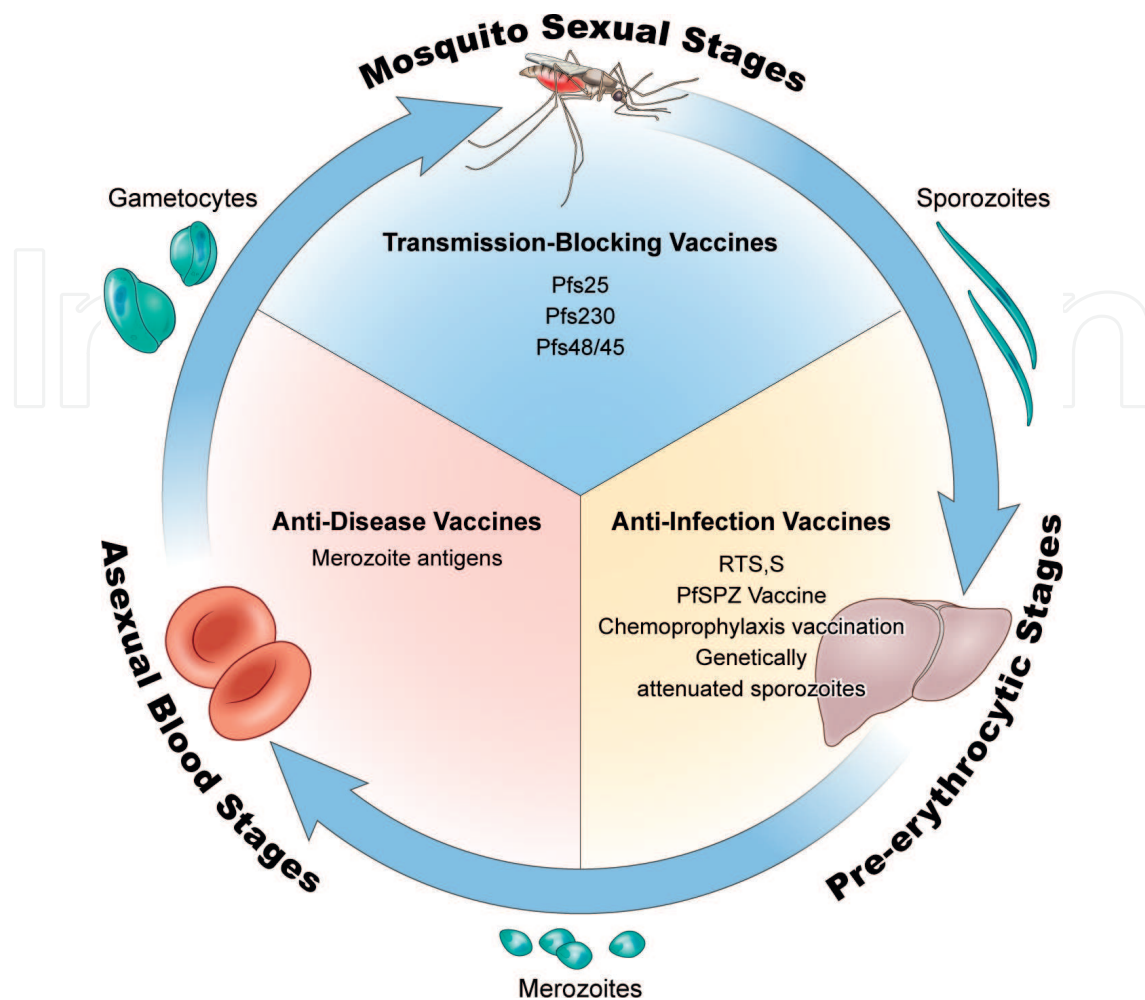
Figure 1. Malaria parasite life cycle and vaccine targets. Transmission-blocking vaccines are directed against the sexual stages of malaria parasite development in the mosquito, while other stages of the parasite life cycle can be targeted with different vaccine approaches. The vaccine concepts, candidate vaccines, and candidate antigens discussed in this chapter are presented according to their targeted stage of the parasite life cycle, as well as their anticipated biological effects: transmission-blocking, anti-infection, and anti-disease effects. Illustration by Alan Hoofring, Medical Arts Design Section, NIH.

deaths, according to prevalence-based predictive models [9–11]. The complex malaria parasite lifecycle (**Figure 1**) offers several stages that can be targeted by various vaccine strategies, which in combination may interrupt transmission.

4. Benefits of malaria transmission-blocking vaccines

Current malaria vaccine approaches target various parasite lifecycle stages including liver and blood stages in the individual and sexual stages in the mosquito (**Figure 1**). Liver stage vaccines, best typified by whole sporozoite (SPZ) vaccines that induce sterile protection [12], presumably act through T cell responses [13] and possibly antibodies and prevent progression of liver stage infections to blood stage parasitemia. Blood stage vaccines on the other hand

confer protection that reduces malaria episodes, disease severity, and/or parasitemia. Additionally, immunity against VAR2CSA, a member of the *P. falciparum* erythrocyte membrane protein 1 family that binds to chondroitin sulfate A, may prevent placental malaria [14].

Vaccines that target the sexual stages, known as TBVs, are the focus of this chapter. TBVs do not directly protect immunized individuals but specifically block onward transmission by preventing mosquito infection. TBVs utilize antigens expressed during mosquito parasite stages (gametocytes, gametes, zygotes and ookinetes) to induce functional antibodies that attack the parasite in the mosquito and impair its viability, inhibit its development, or impede its interaction with the mosquito midgut. The effector antibody responses involved in these types of vaccines include neutralization and complement-mediated lysis. A broader concept coined as a Vaccine to Interrupt Malaria Transmission (VIMT) by the Malaria Eradication Research Agenda (MalERA) includes not only TBVs but also pre-erythrocytic and blood stage vaccines, as well as mosquito molecules involved in parasite development [15] such as *Anopheles gambiae* aminopeptidase 1 (AnAPN1), carboxypeptidase, and saglin.

Ideally, TBVs will elicit effective antibodies that prevent malaria parasite development in mosquitoes after uptake of blood meals. This will reduce the number of circulating infectious mosquitoes below a threshold that sustains transmission. TBVs are among the tools being encouraged for use during pre-elimination and elimination phases of malaria eradication according to malERA [15] and could be an effective alternative or adjunct to vector control. Compared to vector control interventions, TBVs are ecologically safer, cost-effective, and readily enable high coverage of populations.

Most TBV antigens are genetically conserved, which may be due to limited immune pressure. The effect of immune pressure exerted by TBV against the parasite remains unknown and will need to be monitored in future. Notably, sexual stages are critical for the generation of parasite genetic diversity and regulation of parasite virulence, hence the effects of TBVs on these phenomena also warrant monitoring. In addition, malaria parasites experience a considerable population bottleneck in the mosquito for only a handful of parasite zygotes progressing to oocysts on the mosquito midgut. Altogether, while these observations make the mosquito phase an attractive target for vaccine development, much remains to be done to achieve implementable and effective TBVs.

5. Current status of malaria vaccine research

The development of effective vaccines against eukaryotic organisms is far from easy and has been particularly difficult for *P. falciparum*, a protozoan parasite characterized by three genomes (nuclear, mitochondrial, and apicoplastid), an adenine-thymine rich (~80%) nuclear genome [16] encoding >5000 genes, and a complex lifecycle involving several developmental stages between vertebrate and invertebrate hosts. Malaria vaccine development has been hampered by several factors during a century of effort, including the genetic diversity of *P. falciparum*, complexity of its biology, and difficulty obtaining long-lasting effective immunity. Interestingly, adults living in hyperendemic settings are continuously exposed to infective

mosquito bites and naturally acquire immunity that controls parasitemia and reduces clinical episodes of malaria over time. Responses against some parasite proteins have been associated with this natural protection, which makes them promising vaccine targets [17].

Today, the most advanced malaria vaccine is RTS,S, a pre-erythrocytic stage vaccine consisting of a virus-like particle (VLP) that displays hepatitis B surface antigen alone (S) and fused with a *P. falciparum* circumsporozoite protein fragment containing its central repeats and T cell epitopes (RTS). RTS,S has completed Phase III clinical trial (vaccine given to thousands of people and tested for efficacy and safety) and showed an efficacy of 51.3% (95%CI, 47.5–54.9) against clinical malaria in 5- to 17-month children over 12 months after three doses of the vaccine. A fourth dose was required to sustain protection over longer periods [18]. RTS,S is currently in pilot implementation studies involving 360,000 young children, expected to be given the vaccine in Ghana, Kenya, and Malawi. Although this represents important progress given the absence of any other human vaccine against a eukaryotic pathogen, more research is needed to develop vaccines that meet the Malaria Vaccine Technology Roadmap goals of 50% efficacy against severe malaria for more than one year and ≥75% long lasting efficacy against clinical malaria. For example, alternative dosages, timing and number of doses, are being evaluated as strategies to improve RTS,S efficacy [19, 20].

Attenuated whole SPZ vaccines have shown high-level sterile protection (>90%) against homologous challenge in early clinical trials [21] and thus have been heralded as a promising malaria vaccine approach. The concept of immunization using the whole SPZ was first attempted in 1910 by the French scientist Sergent using an avian model of malaria [22]. Several decades later, protective immunity was induced in mice following inoculation of X-irradiated SPZ of *P. berghei* [12]. In 1973, this approach was shown to be protective in humans, using X-irradiated SPZ of *P. falciparum* to vaccinate, followed by challenge with the non-irradiated homologous strain delivered by mosquito bites [23]. More recently, inoculation of non-attenuated fully infectious SPZ from chemo-sensitive strains along with administration of effective antimalarial drugs, known as chemoprophylaxis vaccination, was shown to induce sterilizing immunity [24]. Immunity induced by chemoprophylaxis vaccination is dose-dependent and requires substantially smaller SPZ inocula compared to irradiated SPZ [25].

Finally, genetic attenuation of parasites through the deletion of liver developmental stage-specific genes by homologous recombination is also being pursued to generate whole SPZ vaccines [26]. Numerous technologies may generate genetically attenuated parasite vaccines, including flippase (Flp)/Flp recognition target, Cre/loxP recombination, zing-finger nucleases, and the clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated protein 9 (CRISPR/Cas9) system [27–30]. Genetic attenuation enables generation of parasites that arrest at late liver stages, exposing a broader liver stage-specific antigen repertoire to the immune system over a longer duration. However, genetic attenuation can be incompletely effective for preventing breakthrough to blood stage parasitemia, and this needs to be monitored carefully in clinical studies. Further, the requirement for mosquitoes to deliver SPZ vaccines had been considered as an insuperable obstacle to development of a whole SPZ vaccine for mass immunization. This obstacle has been partially overcome by the production of purified, aseptic, and cryopreserved SPZ for syringe injection by Sanaria Inc. [31].

The blood stage is another important focus for malaria vaccine research, as this stage is responsible for the clinical manifestations of malaria. People living in endemic areas are repeatedly exposed to blood stage parasites and acquire protective antibodies over years that control parasitemia and prevent disease; consequently, a blood stage vaccine can be composed of antigens targeted by naturally acquired immunity that prevent clinical episodes of malaria [17, 32]. For example, immune responses to combinations of merozoite antigens were associated with 100% protection against clinical episodes of malaria in Kenyan observational cohorts [17]. Unfortunately, the efficacy of merozoite antigen vaccines in interventional trials has been poor, limited in part by antigenic diversity, which must be overcome for effective strain-transcending vaccines [33, 34].

6. Transmission-blocking vaccine (TBV) development

The first demonstration of antibodies that prevented mosquito infection was reported in 1958 using the avian species *Plasmodium gallinaceum* [35]. However, it remained until 1976 for studies to show that such antibodies might recognize gamete proteins and therefore act against the parasite in the invertebrate rather than vertebrate host [36, 37]. These gamete proteins were subsequently characterized to be P230 and P48/45, and later the zygote/ookinete surface proteins P28 and P25 were shown to be TBV targets; "P" refers to *Plasmodium* (the antigens have homologs in all *Plasmodium* species to date), and the number refers to their molecular weights on SDS-PAGE [38, 39]. P28 and P25 are paralogs, most abundant on the surface of zygotes and ookinetes, glycosylphosphatidylinositol (GPI)-anchored, and involved in ookinete formation. Today, these four parasite proteins represent the leading TBV candidates. Ookinete-secreted proteins have also been identified as targets for TBVs, including chitinase 1, von Willebrand factor-A domain-related protein, thrombospondin-related anonymous protein-related protein, membrane-attack ookinete protein, secreted ookinete adhesive protein (SOAP), and cell-traversal protein for ookinetes and sporozoites (CelTOS) [40, 41].

TBVs that have reached human clinical trials include only Pfs25 and its *Plasmodium vivax* ortholog Pvs25, and Pfs230. Early clinical trials of Pfs25 and Pvs25 yielded poor results due to either poor production of antibodies with transmission-blocking activity or to significant reactogenicity attributed to adjuvant formulations [42–44]. These challenges have been addressed by advances in vaccine expression systems, delivery platforms, and adjuvant formulations. Production of recombinant TBV antigen has been assessed in numerous systems, including *Escherichia coli*, *Saccharomyces cerevisiae*, *Pichia pastoris*, and baculovirus/insect cells, to yield better-folded proteins that are stable in solution and recreate conformational epitopes. Pfs230 and Pfs45/48 vaccines in particular are hampered by difficulty in expressing them in their appropriate conformations. To overcome this, research has focused on the expression of immunogenic fragments rather than full-length proteins [45, 46].

Several approaches to vaccine particle preparation have also been pursued to increase immunogenicity. These include conjugation to carriers (such as *Pseudomonas aeruginosa* exoprotein A (EPA) [47] and bacterial outer membrane protein complex (OMPC) [48]) or fusion to partners that complex to generate particles (such as C4 bp oligomerization domain (IMX313) expressed

in *E. coli* [49, 50] or modified lichenase carrier (LiKM) produced in *Nicotiana benthamiana* [50]). Viral vector vaccines, such as Chad63/Modified Vaccinia Ankara, are also being assessed to improve immunogenicity [49].

Adjuvants, such as Alhydrogel® and Montanide®, have been used for clinical trials of TBVs with reactogenicity issues observed with both; however, recent trials of Alhydrogel®-formu-lated TBV have demonstrated good safety and reactogenicity profiles. Recently, GSK®'s lipo-somal adjuvant AS01 has been considered for TBVs. AS01 incorporates the TLR4 ligand MPL and the saponin derivative QS-21, and because AS01 is used for formulating the pre-erythrocytic vaccine RTS,S, this would simplify future efforts to combine products.

TBV candidates that are in clinical and preclinical developments are summarized in **Table 1**. Human studies of Pfs25 and Pvs25 showed that priming doses of the vaccines do not induce detectable antibody levels [42, 51]. Antibody production is measurable after the first boost and then rapidly declines; additional boosts are required to retain antibody titers. However, the raised antibodies have been proven to be functional, i.e. capable of reducing oocyst formation in mosquito-feeding assays, and this activity strongly correlates to antibody titer [42, 51]. In addition to Pfs25/Pvs25 candidates, Pfs230 conjugated to EPA and adjuvanted in Alhydrogel® or AS01 has advanced to clinical trials for evaluation either alone or in coadministration with Pfs25 (https://clinicaltrials.gov/, trials NCT02334462 and NCT02942277).

Vaccine candidate	Type	Stage of development	Clinical trial identifier or reference number
Pfs25M-EPA/AS01 and/or Pfs230D1M-EPA/AS01	Subunit vaccine	Phase 1	NCT02942277
Pfs230D1M-EPA/Alhydrogel® and/or Pfs25 EPA/Alhydrogel®	Subunit vaccine	Phase 1	NCT02334462
Pfs25-EPA/Alhydrogel®	Subunit vaccine	Phase 1	NCT01867463, 51
Pfs25 VLP-FhCMB	VLP vaccine	Phase 1	NCT02013687
ChAd63 Pfs25-IMX313+/-MVA Pfs25-IMX313	Viral vector & nanoparticle vaccines	Phase 1	NCT02532049
Pfs25 & Pvs25/Montanide ISA 51	Subunit vaccine	Phase 1	[43]
Pvs25H/Alhydrogel®	Subunit vaccine	Phase 1	[42]
Pfs25-Pfs25	Conjugate vaccine	Phase 1	NCT00977899
Plant-Produced Pfs230 LiKM	Subunit vaccine	Preclinical	[57]
Pfs48/45	Subunit vaccine	Preclinical	[58, 59]
Pvs48/45	DNA vaccine	Preclinical	[60]
Pvs47	DNA vaccine	Preclinical	[60]
Pfs28	Subunit vaccine	Preclinical	[61, 62]
PfHAP2	Subunit or viral vector vaccine	Preclinical	[55, 63]
AnAPN1	Subunit vaccine	Preclinical	[59, 64]

Table 1. Transmission-blocking vaccine (TBV) candidates in clinical and preclinical developments.

7. Field studies of TBVs and Malian experiences

Field clinical trials are a major component of TBV development. TBV candidates are generally discovered in laboratories in the North with extensive infrastructure for modern biological sciences to conduct discovery research. After passing preclinical evaluations, TBV candidates must be tested in humans to qualify as viable vaccines. Safety and tolerability of the products are assessed first, generally in malaria-naïve individuals in non-endemic countries during a first-in-human phase 1 study. If the product meets acceptable safety and tolerability criteria, then it advances to Phases I, II, and III field clinical trials, which often means evaluation in malaria-endemic settings. Field studies are essential for assessing interruption of malaria transmission in the communities living in malaria-endemic areas.

A field clinical trial is not just a simple study but rather a multifaceted activity that builds on strong partnerships between research institutions and affected communities. Various capabilities are required for successful clinical trials, including confidence-based collaborative research teams, facilities, equipment, written procedures, training programs, community engagement, collaboration with ethics review committees, and collaborations with health and political authorities. The partnerships include vaccine inventors, developers, sponsors/funders, and institutions that have appropriate capacities and experience in conducting field clinical trials of malaria vaccines.

The main components of a TBV field clinical trial comprise immunization of study volunteers with prime and boost doses, intensive safety follow-up and reporting, mosquito-feeding assays, and the measurement of antibody responses using enzyme-linked immunosorbent assay (ELISA) for titers and standard membrane-feeding assay (SMFA) for activity. The immunization of volunteers is a major event that involves professionals with sundry expertise. The professionals include clinicians who assess volunteers for inclusion/exclusion criteria and monitor their health after receiving the vaccination, pharmacists who manage the randomization list as well as vaccine preparation, physicians who administer the vaccines, and intensivists who provide care for any post-immunization emergency. Medical biologists ensure proper biological sample collection, processing, transport, and storage as well as immediate measurement of biological parameters. Medical entomologists perform mosquito-feeding assays and associated dissections for endpoint analysis, and data managers enter and ensure quality of data according to established procedures.

8. Measuring vaccine activity

Unlike other vaccines where controlled human malaria infection (CHMI) studies are useful for assessing efficacy, field assessment of TBV efficacy currently requires mosquito-feeding assays on individuals living in malaria-endemic areas. Several mosquito-feeding assays can be utilized to assess the capacity of vaccine-induced antibodies to interfere with mosquito infectivity including direct skin-feeding (DSF) assay, direct membrane-feeding assay (DMFA), and standard membrane-feeding assay (SMFA). The DMFA entails feeding of laboratory-reared uninfected mosquitoes on venous blood immediately after collection from study participants

(**Figure 2A**). Feeding occurs through various types of membranes, such as pig intestine or Parafilm®, to access infected blood housed in a heated chamber that attracts mosquitoes. This method takes the diversity of infection in the population into account. The standard membrane-feeding assay (SMFA) is the gold-standard technique for functional evaluation of antibodies in TBV studies, given its use of a well-characterized laboratory parasite isolate and mosquito line that lend themselves to standardization. Mosquitoes feed on cultured gameto-cytes together with either volunteer serum or purified immunoglobulin (**Figure 2B**). SMFA is similar to DMFA in the machinery and process for feeding but fails to capture parasite diversity effects on vaccine activity.

In DSF assays, cups of field-adapted, laboratory-reared mosquitoes are fed on the skin of human volunteers to assess the ability of vaccine antibody responses to block malaria trans-mission in near-natural conditions (**Figure 3**). In a recent advance, the Malaria Research and Training Center (MRTC) in Bamako (Mali) and the Laboratory of Malaria Immunology and Vaccinology (LMIV) at NIAID/NIH, in Rockville, MD (USA) have established the infrastructure, logistics, and safety database to support scale up of DSF assays on a community-wide basis (**Figure 3**). These DSF assays use a line of locally caught *Anopheles coluzzii* recently adapted for breeding in a contained insectary. In these studies [52], uninfected mosquitoes (generally 30–60 per assay) are fed on study volunteers on a regular basis during the malaria transmission season, with the expectation that an effective vaccine will reduce the number of infected mosquitoes compared to controls. We believe DSFs, in which insectary-raised clean mosquitoes are directly fed on infected individuals, may be more likely to be predictive of an intervention's impact on transmission than membrane feeds.
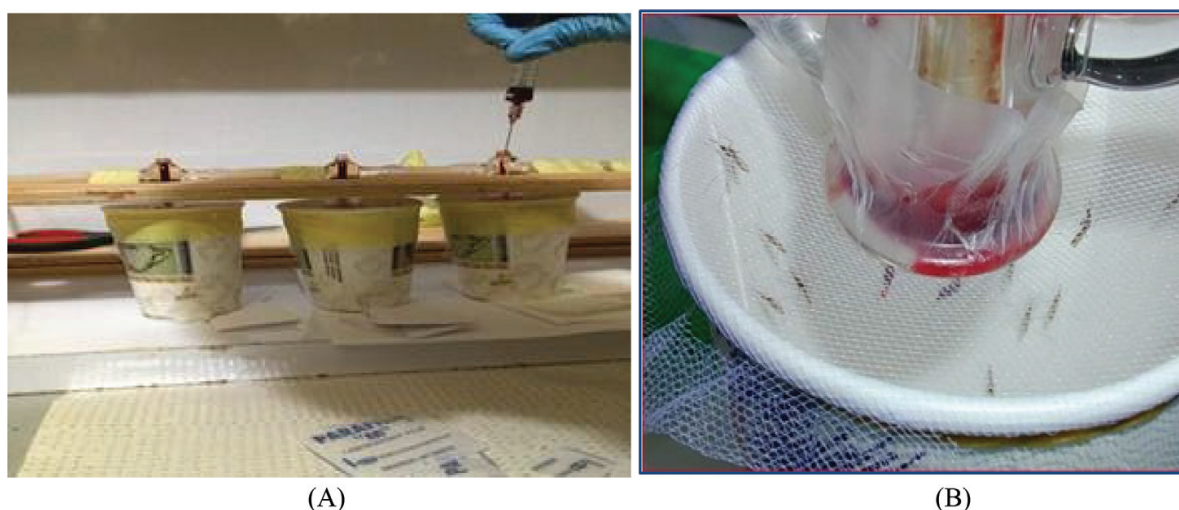

(A)   (B)

Figure 2. Membrane-feeding assays. (A) Overall setup of a direct membrane-feeding assay (DMFA). DMFA was performed at the Malaria Research and Training Center (MRTC), Mali with mosquitoes feeding through a membrane on whole venous blood taken in citrate-phosphate-dextrose or heparin from infected donors. The mosquitoes used were F1 or F2 progeny of wild-caught mosquitoes, or MRTC colony-bred mosquitoes (*Anopheles coluzzii*) maintained for many generations after local capture. The gametocyte source was fresh venous blood collected from infected study volunteers in Mali. (B) Standard membrane-feeding assay (SMFA) showing an individual feeding chamber. SMFA performed at the Laboratory of Malaria Immunology and Vaccinology (LMIV), National Institute of Allergy and Infectious Diseases (NIAID)/National Institutes of Health (NIH), Rockville, MD, USA with mosquitoes feeding through a membrane on laboratory-cultured parasites (gametocytes) suspended in media with immune or nonimmune serum/plasma or IgG. The mosquitoes used were an established laboratory strain (commonly *Anopheles stephensi*).

Figure 3. Direct skin feeds (DSF). DSF was performed at the Malaria Research and Training Center (MRTC), Mali at the study sites. MRTC colony-bred mosquitoes (*Anopheles coluzzii*) raised in containment fed directly on skin of consenting study participants. The gametocyte source was infected study volunteers in Mali. Ethical approvals were obtained from both the NIAID/NIH IRB (National Institute of Allergy and Infectious Diseases/National Institutes of Health, Rockville, MD, USA Institutional Review Board) and the Mali FMPOS (Faculty of Medicine, Pharmacy and Odonto-Stomatology) Ethics Committee.

For all these methods (SMFA, DMFA, DSF), mosquitoes are dissected about a week after feeding for oocyst detection and counting by microscopy. The results of the mosquito-feeding assay allow the calculation of transmission-blocking activity and transmission reducing

activity, which are respectively the ratio of the proportions of infected mosquitoes and the mean counts of oocysts, between test and control mosquitoes.

The measurement of antibody titers during field clinical trials of TBVs (generally by ELISA) is required to assess the immunogenicity of vaccine candidates. Samples are assayed to detect any pre-existing immunity against vaccine candidates, to determine vaccine immunogenicity after prime and boost doses, and to monitor the decay of antibody titers during the follow-up. In addition, antibody titer data can be linked with mosquito-feeding assay data to determine the correlation between antibody production and functionality. Many further investigations can be performed with samples collected from the study volunteers for other exploratory objectives.

9. The Malaria Research and Training Center (MRTC) field trials experience

The achievement of all field activities requires an institution endowed with strong capacities and trained staff. Malaria Research and Training Center (MRTC), founded in 1992 at the Department of Parasitic Diseases Epidemiology based at the University of Sciences, Techniques and Technologies of Bamako (USTTB) in Mali, is a leading institution in conducting field clinical trials of TBVs in Africa. The center is the result of a partnership between the Malian government and US National Institutes of Health (NIH) to build capacity in malaria research and training in Mali. The USTTB collaboration has been designated by NIH as an International Center for Excellence in Research (ICER).

MRTC comprises six equipped and autonomous clinical trial sites located in malaria-endemic Malian villages that fulfill International Conference on Harmonization (ICH) requirements and adhere to Good Clinical Practices (GCP) and a central laboratory in Bamako (the capital city of Mali) where several teams are based. The MRTC infrastructure includes a clinical laboratory certified by the College of American Pathologists and an equipped insectary, among others. In addition, the center has 15 other field sites that host epidemiological studies and other collaborations with external partners.

From 2003 to 2016, MRTC completed 13 asexual stage vaccine clinical trials and one TBV field clinical trial evaluating Pfs25H-EPA/Alhydrogel® (Bancoumana, Mali, from 2013 to 2016, NCT01867463) in collaboration with LMIV at NIH. Field clinical trials of six more candidate TBVs are ongoing: Pfs25M-EPA/Alhydrogel® and Pfs230D1M-EPA/Alhydrogel®, as well as the combination of Pfs25M-EPA/Alhydrogel® + Pfs230D1M-EPA/Alhydrogel® (Bethesda, USA and Bancoumana, Mali; started in 2015, NCT02334462), and Pfs25M-EPA/AS01, Pfs230D1M-EPA/AS01, and the combination of Pfs25M-EPA/AS01 + Pfs230D1M-EPA/AS01 (Bamako, Doneguebougou and Bancoumana, Mali; started in 2016, NCT02942277). The success of these studies depends on MRTC's technical capacities and strength in community engagement. Dynamic MRTC teams execute intense programs of volunteer immunization and thorough follow-up (**Figure 4**), as well as high-throughput processing of samples in laboratories, rearing of mosquitoes, mosquito-feeding assays, dissection of mosquitoes, real-time data entry and cleaning, and constant transportation of persons, samples, and materials between central laboratory and study sites.
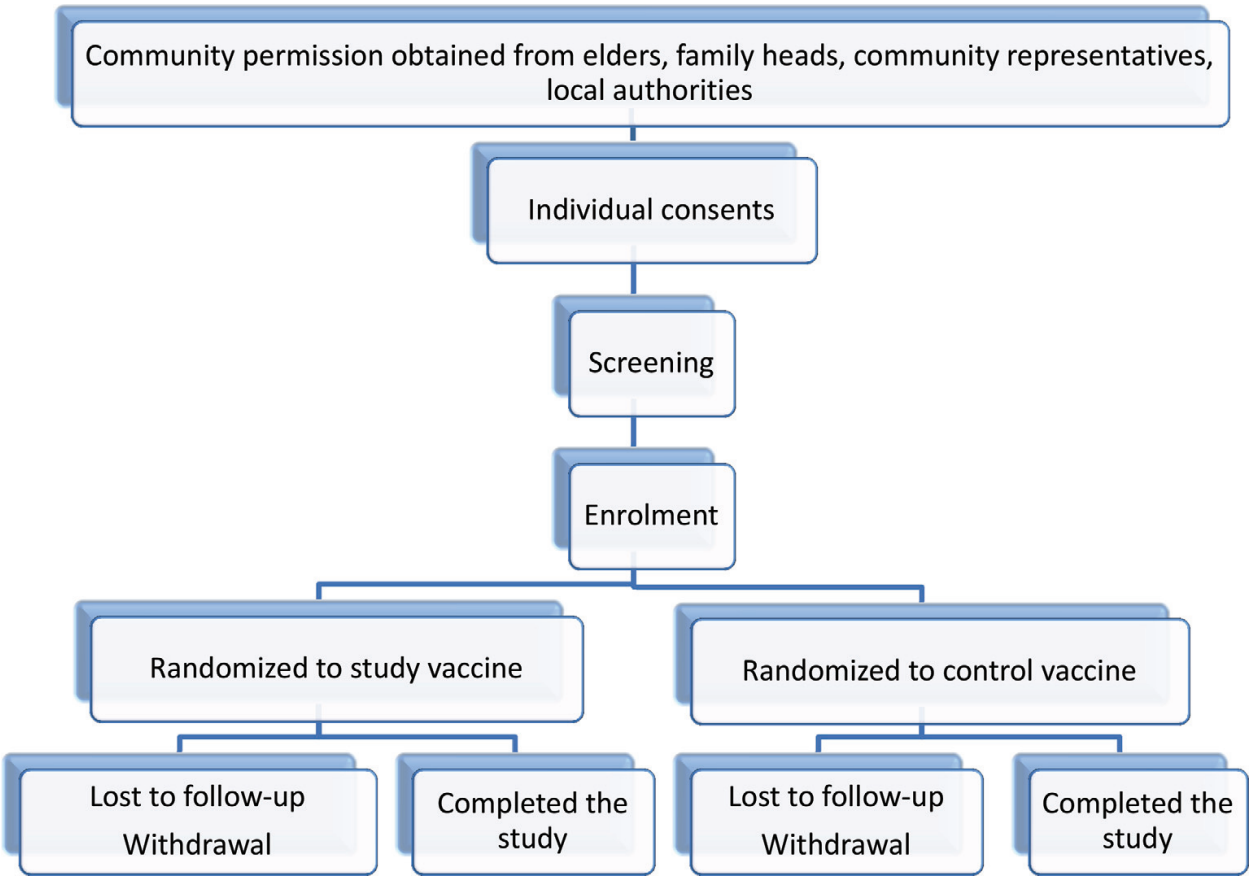
Figure 4. General profile of a malaria transmission-blocking vaccine trial in Mali. This is general study process (flow-chart) followed at the Malaria Research and Training Center (MRTC), Mali in carrying out a malaria vaccine study.

For community engagement, MRTC has built participative, durable, and confidence-based partnerships with communities at and around the study sites. Initially, community leaders comprising village heads, elderly, family heads, women's and youth's representatives, local health providers, and school teachers are consulted for permission to build research facilities and conduct clinical trials in the communities [53] (**Figure 4**). Subsequently, individual consents are obtained from interested volunteers according to international guidelines.

Local residents are fully involved and meaningfully impacted by the research activities. The presence of research teams and facilities, along with successful execution of clinical trials, has contributed to significant improvement of the local healthcare system and even the economy of the host villages. This positive impact has reinforced community confidence towards clinical trials and subsequently strengthened community engagement, positioning MRTC to success-fully conduct TBV field clinical trials in Mali.

10. Bottlenecks and perspectives for TBVs

The future development and potential implementation of current TBV candidates will need to address important regulatory issues. A major issue involves the conceptual framework for a

vaccine that does not directly benefit a recipient, but instead benefits a community only when the proportion of the population that receives the vaccine achieves the threshold needed to reduce malaria prevalence and incidence. This threshold may vary between communities depending on the baseline intensity and ecology of malaria transmission. A TBV can be considered a "vaccine of solidarity," whereby the individual accepts vaccination to contribute to protection of his/her community and thereby ultimately to his/her own benefit. Before any decision to participate, individuals must balance the risks of immunization and the benefits for the community and ultimately themselves. To address this issue during clinical trials, candidate study participants must pass a test of comprehension that indicates the participant's understanding that the TBV has no potential to directly prevent their own infections. At present, there are few data in the literature on individual and community perceptions and understanding of TBV. Education on the mechanisms of action and potential community/individual benefits of TBV will be required before implementation.

Additionally, this community-based approach and vaccine coverage goal sets a high bar to achieve, even for a malaria vaccine. The vaccine needs to be efficacious not only in children but also in the majority of individuals in the community that contribute to transmission. The impact of factors such as malaria exposure, immunodeficiency, coinfection, pregnancy, and age on antibody responses will require careful study after qualification of promising TBV candidates. Thus, further research is needed to understand transmission dynamics within a community, durability and boosting of TBVs, and immunogenicity throughout all individuals within a community.

Furthermore, the DSF assay that experimentally exposes humans to mosquito bites raises ethical concerns and logistical burdens that may impact scientific objectives and study designs, and in some communities, recruitment and retention of volunteers. Mosquitoes used for DSF are reared in controlled and contained conditions to ensure the safety of the volunteers. While DSF has approval by ethical review committees, community perceptions should be considered to reassure participants and promote participant engagement in the process and rationale to prevent loss to follow-up that can compromise trial objectives. Our experience in Mali shows that strong community relationships and individual education in study design and goals have resulted in high participation and retention rates for studies that incorporate DSF assays.

The leading TBV candidates are either not exposed (Pfs25) to the immune system naturally or induce only modest responses naturally, and thus natural boosting may do little or nothing to extend antibody responses. Human antibodies should act quickly before degradation in the mosquito and before parasite traversal of midgut epithelium (~24 h) that limits its accessibility to antibody. The need for TBV to maintain high levels of potent antibody that preclude mosquito infection is the major hurdle for developing TBV. Today, several strategies are being pursued to overcome this hurdle, including the improved adjuvants and delivery systems discussed above. Additional antigens are also under exploration such as Pfs48/45 [54] and PfHAP2 [55] that could expand the portfolio of TBV in future.

As there is no existing human-based intervention that uniquely aims at blocking parasite transmission, such a tool could shift interest toward the interruption of transmission in comparison to the urgent need for interventions that protect and treat children under five and pregnant women. Once TBV tolerability and impact in the field are confirmed in advanced

clinical trials, consideration must be given for how this vaccine should be implemented along-side other malaria control tools. Ultimately, TBVs are envisioned as one tool in the toolbox of interventions, including antimalarial drugs, vector control measures, and other vaccines, that will be required for malaria elimination.

11. Conclusions

Malaria control has improved with the redoubling of efforts and financial resources in recent years, but existing tools will lose their effectiveness over time. The best long-term strategy to address the malaria scourge is its elimination and ultimately eradication, but existing tools are insufficient for this purpose. This gap has been recognized by the WHO Roadmap for Malaria Vaccines that specifically calls for vaccines that can be used for elimination [56]. TBVs prevent human-to-mosquito transfer of parasites and hence are well-suited for use in elimination and eradication programs. Development of TBV has been hindered by the nature of the target antigens, which have been difficult to express in proper conformation and are poorly immu-nogenic, as well as the dearth of resources dedicated to their development. However, increas-ing attention to elimination by policy makers and funding agencies has reignited interest in this research area, and improved platforms for vaccine expression and delivery have yielded promising new TBV candidates that have in some cases advanced to field trials. Collaborative multidisciplinary teams are now rising to the task of testing TBVs in the field, which also require specialized facilities to measure transmission-blocking activity of vaccines. Regulatory issues will need to be addressed as TBVs are developed and implemented, particularly because these products are designed to benefit the community and not the individual directly. How-ever, vaccines have been essential for eliminating or eradicating other infectious agents, and TBVs could be a vital component of a multipronged effort to eradicate malaria from the face of the earth.

Acknowledgements

Acronyms

AnaPN1	*Anopheles gambiae* aminopeptidase 1
AS01	GSK's adjuvant system containing liposome, 3-O-desacyl-4′-monophosphoryl lipid A (MPL) and *Quillaja saponaria* Molina saponin fraction 21 (QS21)
CELTOS	Cell-traversal protein for ookinetes and sporozoites
CHMI	Controlled human malaria infection
CRISPR/Cas9	Clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated protein 9 (Cas9)
DMSFA	Standard membrane-feeding assay
DSF	Direct skin-feeding assay
ELISA	Enzyme-linked immunosorbent assay
EPA	*Pseudomonas aeruginosa* exoprotein A.
EPI	Expanded Programme on Immunization
Flp	Flippase
FMPOS	Faculty of Medicine, Pharmacy and Odonto-Stomatology
GCP	Good Clinical Practices
GPI	Glycosylphosphatidylinositol
GSK	GlaxoSmithKline
ICER	International Center for Excellence in Research
ICH	International Conference on Harmonization
IMX313	C4 bp oligomerization domain
IRB	Institutional Review Board
IRS	Indoor residual spraying
ITN	Insecticide-treated nets
LikM	Modified lichenase carrier
LMIV	Laboratory of Malaria Immunology and Vaccinology
MalERA	Malaria Eradication Research Agenda
MPL	3-O-desacyl-4′-monophosphoryl lipid A

MRTC	Malaria Research and Training Center
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health
OMPC	Outer membrane protein complex
QS21	GSK's *Quillaja saponaria* Molina saponin fraction 21
RTS,S	Hepatitis B surface antigen alone (S) and fused with *Plasmodium falciparum* circumsporozoite protein fragment containing its central repeats and T cell epitopes (RTS)
SDS-PAGE	Sodium dodecyl sulfate-polyacrylamide gel electrophoresis
SMFA	Standard membrane-feeding assay
SOAP	Secreted ookinete adhesive protein
SPZ	Sporozoite
TBV	Transmission-blocking vaccine
TLR4	Toll-like receptor 4
USTTB	University of Sciences, Techniques and Technologies of Bamako
VAR2CSA	Member of *Plasmodium falciparum* erythrocyte membrane protein 1 (PfEMP1) family binding to chondroitin sulfate A (CSA)
VIMT	Vaccine to Interrupt Malaria Transmission
VLP	Virus-like particle
WHO	World Health Organization

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