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Review Article

Malaria vaccine: a current perspective

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Abstract

The observation that inactivated *Plasmodium* sporozoites could protect against malaria is about a hundred years old. However, systematic demonstration of protection using irradiated sporozoites occurred in the nineteen-sixties, providing the impetus for the development of a malaria vaccine. In 1983, the circumsporozoite protein (CSP), a major sporozoite surface antigen, became the first *Plasmodium* gene to be cloned, and a CSP-based vaccine appeared imminent. Today, 25 years later, we are still without an effective malaria vaccine, despite considerable information regarding the genomics and proteomics of the malaria parasites. Although clinical immunity to malaria has been well-documented in adults living in malaria endemic areas, our understanding of the host-immune responses operating in such malaria immune persons remains poor, and limits the development of immune control of the disease. Currently, several antigen and adjuvant combinations have entered clinical trials, in which efficacy against experimental sporozoite challenge and/or exposure to natural infection is evaluated. This review collates information on the recent status of the field. Unresolved challenges facing the development of a malaria vaccine are also discussed.

Key words CSP – malaria vaccine – *Plasmodium falciparum* – *P. vivax* – pre-erythrocytic stage

Introduction

Our relationship with parasites has been a long one on the evolutionary scale. The methods adopted by parasites to thrive and colonize living organisms are truly fascinating. Along with basic features such as fecundity and resistant cyst structures, the parasites exhibit a fine-tuning of modifications in response to the attack by the host immune system. While the host fights the parasites through its armory of immune as well as certain behavioural responses, the parasites appear to use the host immune responses towards quorum sensing, limiting their own number, but surviving. The human malarial parasite, *Plasmodium falciparum*, which appears to have an ancient origin and has evolved in parallel with humans[1], is known to possess a complex arsenal of defences against man, and therefore the efforts to generate an effective malaria vaccine have been fraught with obstacles.

A malarial infection starts when the sporozoite stage of the unicellular protozoan *Plasmodium* is introduced by the female *Anopheles* mosquito in a vertebrate organism during a blood-feed. Some sporozoites find their way to the liver and infect hepatocytes. Inside the hepatocyte the parasite transforms itself and proliferates massively into the merozoite stage that will infect erythrocytes. Subsequent cyclic erythrocytic stages are responsible for the symptoms, complications and fatality associated with malaria. After a few cycles of asexual stages, some of the infected erythrocytes differentiate into gametocytes, and once

ingested by the mosquito through a blood meal, undergo fertilization generating an oocyst. The sporozoites, produced in the oocyst, migrate to the mosquito salivary glands and are then ready to be introduced into the next vertebrate host. Amongst the four *Plasmodium* species that infect humans, *P. falciparum* is the most dangerous in terms of disease pathology.

A good estimation of the current burden of malaria has been difficult, but nevertheless it is apparent that approximately one million persons succumb to malaria every year in Africa[2]. Devising an effective malaria vaccine would certainly help in limiting such morbidity. Over the years, numerous attempts have been made to develop a vaccine against malaria. The possibility of using inactivated sporozoites was first demonstrated in 1910 in avian malaria[3]. It was followed by studies in 1941 that showed immunization with irradiated sporozoites could prevent infection[4]. Besides irradiated sporozoites, the other observation that holds promise for a vaccine comes from the documented 'clinical immunity' observed in adult residents of malaria endemic areas. Several reviews have covered the field of malaria vaccine over the last decade, some of them fairly recent[5-14]. In this article we attempt to summarize where we stand vis-à-vis an effective malaria vaccine. As of now, the antigens that have been postulated to be protective and have been tested at least in the murine model with a parasite challenge are shown in Annexure 1. Of these, those that have gone in some kind of a clinical trial are shown in Annexure 2. The focus is on the *P. falciparum* vaccine. However, a few *P. vivax* candidates, that have made it to the trials, are also included in the Annexures.

Pre-erythrocytic stage (PE) vaccine

Sporozoites constitute the infective stage of the malarial parasite and they are ideally the target for a malaria vaccine. The pre-erythrocytic phase of infection, which lasts for a few days, is particularly an attractive target of protective immunity, since this phase is clinically silent. In the 1960s, a series of experiments by Nussenzweig, Vanderberg and Most[15] systematically established that irradiated sporozoites do confer protection to the respective vertebrate host. However, a large number of mosquito bites or sporozoites are required to produce such a state of immunity. This led to an immediate hunt for protective antigens of the sporozoite, and the circumsporozoite protein (CSP) was identified[16]. At that time recombinant DNA techonology had just taken off and the hepatitis B antigen, expressed in yeast, was already showing characteristics of a promising subunit vaccine[17]. It was anticipated that one had to simply follow a similar path for the CSP antigen to have a successful malaria vaccine.

Circumsporozoite gene from the simian malaria parasite, *P. knowlesi* H-strain was the first malaria gene to be cloned[18] and was also expressed in yeast shortly after[19]. The deduced protein structure showed novel features such as a centrally located immunodominant repeat region consisting of 12 repeats of 12 amino acids each[20]. Although base changes were detected in the repeat region, the 12 amino acid repeat peptides were completely conserved, implying an important role of the repeat peptides[20]. However, it was soon observed that in *P. knowlesi,* and in the related simian and human parasites, *P. cynomolgi* and *P. vivax,* the repeat peptides were conserved within a strain and the entire repeat region could diversify amongst different strains[21-23]. The antibodies generated against one strain did not cross-react with another[24]. Similar diverse repeat regions were found in several malarial antigens and the mechanism of generation of such diverse repeats was attributed to recombination and spread of such recombined DNA segments during replication[25]. However, *P. falciparum* CSP gene was found to contain a 4-amino acid repeat peptide (NANP)[26,27], the presence of NANP was confirmed in several strains examined, albeit with minor variations. The first malaria vaccine trial based on this repeat region $(NANP)_n$ was conducted in 1987[28,29].

The vaccine failed and the failure was attributed to the lack of a T-cell epitope and subsequently two dominant T-cell epitopes were included in the vaccine[30]. The MHC restriction of response to $(NANP)_n$ and the demonstration of the fact that irradiated sporozoites were generating a T-independent response to the NANP repeats, highlighted the problems of using the NANP region for an efficacious vaccine[31,32]. Nevertheless, $(NANP)_n$ remains the most prevalent antigenic domain used for vaccination as also for the evaluation of the immune response against sporozoites in vaccinated individuals.

The confluence of biology and technology has made CSP the most widely studied vaccine target. CSP is the most abundant sporozoite antigen, and the most easily assayble one. Through knock-out studies it was demonstrated that it is an essential structural protein and that the absence of this protein blocked development in the mosquito stages[33]. However, the CSP-domain that interacts with the hepatocytes is a highly conserved cell-adhesive sequence in the carboxy-terminus of the protein with similarity to the type I thrombospondin repeat (TSR)[34]. Such a conserved domain may undermine its value as a vaccine target. Subsequently, other sporozoite antigens such as thrombospondin-related adhesion protein (TRAP) and certain liver stage antigens were identified and current vaccines against the PE stages consist of epitopes from a combination of antigens (CSP, TRAP and some liver-stage antigens) (Annexure 1). It is interesting to note that $(NANP)_n$ domain of the CSP is a constituent of most of these subunit compositions.

There have now been numerous efficacy trials of various combinations of the antigen formulations (Annexure 2), of which the RTS,S has just concluded a Phase III trial[35-37]. The vaccine efficacy, which is estimated based on the time taken by the subjects to show first infection post-immunization, was 34% (for Gambian adults) and 45% (for Mozambican children). A substantial reduction in the incidence of severe malaria (57%) was also recorded for the children, although the confidence intervals were wide. However, the cumulative numbers of control and vaccinated volunteers who developed parasitaemia were not significantly different after 4–6 months, indicating that the protective effect is short lived. Is this protection due to specific immune responses to the parasite antigen? A recent study using genetically engineered sporozoites with heterologous CSPs has shown that cross-protection is observed without any cross-reactive immune responses to the two CSPs[38]. In most of the vaccine trials also the immune response to CSP did not correlate with protection, excepting for the latest trials on infants from Mozambique using RTS,S/AS02D[39]. This phase I/IIb double-blind randomized trial of 214 infants showed 68 first or only *P. falciparum* infections; 22 in the RTS,S/AS02D group and 46 in the control group, and the adjusted vaccine efficacy was estimated to be 65.9% (95% CI: 42.6–79.8%, $p < 0.0001$)[39]. However, even in this trial the point prevalence of infection at study month 6 was neither different between the two groups (5% in the RTS,S/AS02D group *vs* 8% in the control group, $p = 0.536$), nor were there any differences between mean parasite densities.

Asexual stage vaccines

For a person naïve to malaria, clinical disease is concomitant with the occurrence of erythrocytic stages of *Plasmodium* in the blood. Vaccination against the asexual stages of *Plasmodium* is therefore not an option for the prevention of malaria but more towards diminution of the severity of the disease.Such a 'clinically immune' state is observed in adults resident in malaria hyper- or holo-endemic regions and is termed 'premunition'. It has been demonstrated that administration of gamma-globulins from such malaria immune adults results in the clearance of parasites in the susceptible patients, establishing the therapeutic potential of such antibodies[40,41]. Immunoglobulin G subtype[42] and monocytes[43] have been proposed to play important roles in such a protection.

For an asexual stage malaria vaccine, the impetus came from the establishment of parasite culture by Trager and Jensen[44] and peptide biology. Using peptide fractions from cultured parasites, four synthetic peptides were identified which resulted in a strong immune response in murine models and a formulation of these synthetic peptides gave birth to the first asexual stage vaccine SPf66[45]. It moved rapidly from primate studies to clinical trials[46,47]. However, the results were variable in Africa (efficacy of 2 to 30%). A systematic trial in Thailand failed to show any efficacy in 1996[48], and further vaccine trial has been abandoned by WHO[49]. The vaccine is now being resurrected with new adjuvants and is in the Phase I trial (Annexure 2).

Dominant surface antigens of the merozoite stage have been considered potential vaccine candidates, especially if they can be used with PE stage antigens. Amongst the first to be studied as vaccine candidates were merozoite surface protein 1 (MSP-1) and apical membrane antigen-1 (AMA-1). Affinity-purified native MSP-1 was tested on *Aotus* monkeys and complete protection against asexual stage challenge was observed[50]. It was established that MSP-1 is processed in the parasite and the gene for the precursor MSP-1 protein was the first of the merozoite protective surface antigen gene to be cloned[51]. Although the protein was found to be polymorphic, the carboxy-terminal 42 and 19 kDa fragments are conserved and protective, and these domains form a part of several vaccines (Annexure 2). The AMA-1 protein was first identified as the 66 kDa protective protein from *P. knowlesi*[52]. The gene was then cloned and found to be a conserved gene (and protein) in *P. falciparum*[53]. The identification of the protective protein MSP-3 was somewhat different, since antibodies to this protein did not directly inhibit parasite invasion but used an effector mechanism of antibody-dependent cellular inhibition through monocytes[42]. Other protective proteins of this stage that have made it to the vaccine trials are EBA-175, MSP-2, GLURP, RESA and SERA (Annexures 1 and 2). Apart from merozoite surface proteins, parasite proteins present on the erythrocyte surface, such as PfEMP1, have also been considered for a place in the subunit vaccine. However, due to enormous antigenic variations, these would not constitute potential vaccine candidates[54]. The one exception may be Var1CSA and Var2CSA members of PfEMP1 family that are being considered as candidates for use in a vaccine for pregnancy-associated malaria (PAM)[55].

Malaria vaccine for pregnant women: Although individuals living in malaria endemic areas achieve 'clinical immunity' by the time they reach sexual maturity, the one exception to this rule is pregnancy. PAM is an important cause of maternal and perinatal morbidity and mortality in endemic areas. Pregnant women are more susceptible to malaria than non-pregnant women, and this susceptibility is greatest in first and second pregnancy. Central to the pathogenesis of *P. falciparum* infection in pregnancy is the ability of infected erythrocytes to accumulate in the maternal vascular area of the placenta. Trophozoite and schizont stages of the parasite display this ability to sequester in the placenta. Chondroitin sulphate A (CSA) has been consistently identified as the dominant placental adhesion receptor, although the possibility of the existence of additional receptors cannot be ignored[56]. The first direct evidence that the surface molecules expressed on placenta dwelling infected erythrocytes are likely to be targets of protective immunity came from the work in Duffy's laboratory[57]. They showed that serum IgG from multigravidae exposed to *P. falciparum* could substantially inhibit the adhesion of infected erythrocytes from pregnant women to CSA. The strong negative association between gravidity and susceptibility to malaria in pregnancy suggested that the parasite protein(s) was pregnancy-specific and highly immunogenic, raising hopes for new intervention strategies against PAM. It was subsequently shown that Var1CSA and Var2CSA, members of the highly polymorphic PfEMP1 family encoded by the *var* genes, were candidates involved in CSA-adhesion[55,56]. Of

the two, Var2CSA seems to be the most promising. It is relatively conserved between clones, its transcription is upregulated in parasites from infected placentas, and disruption of the gene abrogates CSA-binding phenotype. Current strategies are focused on expressing these proteins as recombinant antigens. However, the absence of an animal model for *P. falciparum*-associated malarial infections presents some unique hurdles to the development of such vaccines. Even human safety and immunogenicity testing in the target population, a crucial step in any vaccine development, can raise some thorny ethical issues.

Autoimmunity, conserved proteins, anti-toxic immunity and malaria vaccine: Attention was drawn to the infrequent occurrence of autoimmune disorders in tropical Africa, while the susceptibility to autoimmune disorders amongst Africans living in North America was found to be enhanced[58]. Greenwood *et al*[58] attributed this difference to the exposure of the people resident in tropical Africa to various parasitic diseases including malaria, and it was postulated that autoantibodies present in such adults could protect against malaria. The autoantibodies found in clinically protected persons are similar to those present in disorders such as SLE, rheumatoid arthritis, Sjogren's syndrome, polymyositis, scleroderma, Hashimoto's thyroiditis, etc. These can bind double and single stranded DNA, erythrocytes, immunoglobulins, ribonucleoproteins and enolase[59-61]. However, anti-thyroglobulin antibodies, autoreactive to B-cells and found in normal persons, are not enhanced in such a population[62], indicating that it is not a matter of random non-specific polyclonal B-cell activation.

Can some of these autoantibodies provide protection against malaria? We have demonstrated that anti-ribosomal protein (P-proteins) and anti-enolase antibodies do confer protection against malaria[61,63], as also the fact that P-proteins and enolase do get translocated to cell surface[61,64]. Since anti-P-protein antibodies are known to cross-react with dsDNA[65], we can presume that anti-dsDNA antibodies may also confer protection. It is important to note that the titers of these antibodies, though widespread in frequency amongst malaria immune adults[66], are about 100 to 10,000 fold lower than that present in autoimmune patients[67]. Thus, the generation of low levels of several such cross-reactive autoantibodies may cumulatively protect against malaria. If conserved antigens are to be used in a vaccine, one has to identify and use peptide regions which are exclusively present in the parasite, or one has to understand and mimic the controlled low level response to these epitopes. So far vaccines are optimized for a maximum immune response, and we seem to lack the knowledge to generate limited immune responses.

Can we generate a vaccine that will take away the toxicity/severe pathology that occurs amongst a fraction of malaria infected persons? Although the mechanism(s) that cause the severe pathology is poorly understood, one of the parasite specific toxic components is proposed to be the glycophosphatidylinositol (GPI) moiety of *P. falciparum*[68,69]. Mice immunized with GPI glycan were protected against severe disease conditions such as blood acidosis, pulmonary oedema, vascular occlusion by macrophages and cerebral deaths, without any effect on the parasite growth or burden[69].

It is now well documented that malaria parasite causes immunosuppression, and that innate immunity plays a major role in clinical immunity to malaria[70,71]. With the possibility that the parasite GPI and hemozoin may act as the ligands for the 'pattern recognition receptor TLRs', synthetic parasite specific GPI and hemozoin should find a significant place in a malaria vaccine.

Transmission blocking vaccine

Mosquito stage transmission blocking (MSTB) or transmission blocking vaccines (TBV) is anti-mosquito stage vaccine that targets antigens on gametes, zygotes or ookinetes. This strategy can be used in

malaria control due to the particular biology of plasmodia, where transmission-inducing and pathology-inducing parasite forms occur in two different hosts. The idea for TBVs emerged from the 1976 observations of Gwadz[72]; and Carter & Chen[73] who showed that antibodies elicited by gametocytes from the avian malarial parasite, *P. gallinaceum* were capable of killing the emerging gametocytes – not in the avian host but in the mosquito vector.

The ultimate goal of TBVs is the interruption of malaria transmission from human to mosquito populations through prevention of parasite development in the mosquito midgut. Antibodies generated in the host as a result of vaccination may kill the gametocyte within the host or get ingested with the gametocytes and kill the gametes when they emerge from the human host red cell in the mosquito midgut. Unlike vaccines against the other stages of the parasite, antibodies elicited by TBV kill the parasite *outside* the person immunized. Since the hepatic or blood-stage cycle is not targeted, these vaccines are not expected to protect the individual who is vaccinated but instead protect people who live in the immediate area. These vaccines are therefore often called 'herd immunity' or 'altruistic' vaccines. Malaria transmission pattern is determined by the interaction of the human and mosquito reservoir. Transmission is generally focal, because of the localized nature of the breeding sources of the mosquitoes and their limited dispersal range — 1 to 2 km from a breeding site[74]. Hence, altruism is, in practice, often limited to the (extended) family, since infections are often transmitted at household level.

Although a number of parasite proteins have been identified as potential antigens for TBV[75,76], of particular interest are *P. falciparum* Pfs48/45 and Pfs230 and their *P. vivax* orthologs expressed on both macrogametes and microgametes (Annexures 1 and 2). Molecules, such as Pfs48/45, that are expressed only in mosquitoes offer some special advantages for vaccine development. Due to lack of immune selection pressure they may be expected to have little sequence diversity. On the flip side, for such antigens that are never expressed in the human host natural boosting will not occur and the vaccine alone will have to generate long-lasting and effective antibody levels.

The efficacy of the TBV will be dependent upon the proportion of gametocyte carriers immunized in a local transmission area. For any success of TBV vaccination campaigns, a high proportion of carriers would need to be vaccinated and equally importantly, a high proportion of these would need to achieve effective immunity for TBV vaccination campaigns to be successful. Since they are intended to protect communities from infection rather than individuals from the disease, the development of these vaccines faces the added hurdle of conducting Phase III trials to assess impact on the reduction in the incidence of malarial infections, i.e. incidence of clinical cases or parasite prevalence in a TBV-vaccinated community. TBVs will be primarily useful to people living in malaria-endemic or -epidemic regions. They will be of greatest use when considered as part of a multi-stage vaccine strategy or as a part of concerted multi-pronged malaria control strategy.

Whole organism vaccine

Sporozoites: The 'subunit' vaccines that target the PE stages were developed in the hope of reproducing the immunity generated by irradiated sporozoites. But most of these vaccines, which deliver one or a few parasite antigens, induce only partial protection with rapidly fading immune responses[77]. The mechanism(s) by which irradiated sporozoites induce protective responses is as yet unclear. Over the years, studies using irradiated sporozoites have given us some valuable insights into the immune responses to the PE stages of the parasite. Murine models have been the most studied and in these systems, both the T-cells (mainly $CD8^+$) that target intra-hepatocytic stages, and antibodies that recognize antigens on the

sporozoite surface and block sporozoite invasion, appear to be important for protection. In addition, the proteins interferon-g, interleukin-12 and nitric oxide also seem to be critical[78].

It is now known that irradiated sporozoites do penetrate hepatocytes and begin intracellular development in the parasitophorous vacuole, but subsequently stop growing. Irradiated parasites persist in the hepatocytes for up to six months in rats and mice. Eradicating these parasites by chemotherapy abrogates protection in these rodents[79], suggesting that continued synthesis of parasite antigens is required for maintaining protection. Thus, one is perhaps back to square one in the sense that the irradiated sporozoites might constitute the best PE vaccine. Indeed, an assay of liver burden of parasites in the murine model demonstrates that the best reduction is obtained with irradiated sporozoites, and the inhibition is severalfold higher than any of the other formulations[80]. The generation of sporozoites has been refined and recently it has been shown that irradiated sporozoites of *P. falciparum* can generate strong, strain independent protection for at least 10 months in more than 90% of human recipients[81]. Large scale production of sporozoites may lead to the development of an effective sporozoite vaccine. However, the radiation has to be just right; too much will render the vaccine ineffective, too little may result in some parasites remaining virulent and causing the disease rather than protecting against it.

One way to circumvent the above problem would be to have genetically modified sporozoites, which can infect the hepatocytes, but get developmentally blocked post-infection. Mueller *et al*[82] have developed such parasites by knocking out the *UIS3* gene in *P. berghei*, thus constructing the first *Plasmodium* parasite that can penetrate liver cells but not develop further. Such genetically modified sporozoites would be safer than irradiated sporozoites, provided that the modifications are not easily reversed or suppressed through possible intra- or extragenic mutations.

Erythrocytic stages: For blood stage vaccines, vaccination with low numbers of infected red cells is envisaged. Recently, it has been shown that repeated infection of naïve human volunteers with as few as 30 infected red cells, followed by drug treatment, could protect against challenge with a homologous strain of *P. falciparum*[83]. This immunity was predominantly mediated through proliferative T-cell responses, nitric oxide synthase activity and interferon-g production in the absence of antibodies. Subsequently, heterologous protection was demonstrated using a mouse model[84]. A major advantage of this method is that immunity is generated to a range of parasite antigens in a natural setting. However, the dose of the infected red cells need to be worked very carefully, and the concerns regarding the use of blood products from humans, potentially transmitting some as yet unidentified pathogen(s), remains paramount.

Challenges for an effective malaria vaccine

Lack of good animal models for testing of human malaria vaccines, the difficulties in evaluation of efficacy of the vaccine in endemic areas, and the lack of understanding of the immunosuppressive mechanisms of the parasite are the foremost reasons for the failure of an effective vaccine. The use of murine models to demonstrate robust protection has failed to stand true in most human trials. One of the possible reasons for the same is because we use unnatural rodent hosts, and not the natural host (tree shrews) of the murine malaria[85].

Do we have the resources to take empirical approaches to this task? Questions have been raised against extensive field trials for malaria vaccine being conducted with subunit combinations generated mainly in murine models[6]. For some antigen preparations the time taken to go to trials has taken much longer than would have been anticipated, given the observation that vaccination achieved promising protection levels in chimpanzees which are closer to human subjects[86]. For many malarial surface antigen domains,

the usage of correct conformation of the protein regions is known to be extremely important and therefore GMP production of such large numbers of correctly folded complex of antigens is a challenge. Novel concepts of DNA vaccine have been implemented in clinical trials in sheer desperation, although such methods have not been established with any other clinical vaccine. RTS,S-the vaccine that appears to be the most promising may still have problems of interpretation, since certain subjective cutoffs have been used to compute a quantitative efficacy[36].

Perhaps because of the long line of clinical trial failures, at present there appears to be a tilt towards whole organism vaccine. Even though it may be possible to generate large number of irradiated or genetically modified sporozoites, concerns regarding the safety of injecting humans with parasites that have been grown in human RBCs and mosquito cells will remain. There is always the possible risk of unidentified pathogens being delivered with the vaccine. Human erythrocytes are the primary source for clonal amplification of genetically modified parasites as of now. Circumventing this phase of growth does not appear to be possible in the near future.

Even if suitable steps are taken towards the generation of 'safe sporozoites', it is not clear that the children in endemic areas will benefit. The load of parasites to which children are exposed to in endemic areas is already quite large (inoculation rate of >100 per year). Foetal sensitization also occurs for malaria, and children are found to carry detectable loads of parasite[87,88], indicating that the exposure to low levels of parasites does not necessarily protect. For most vaccines, Phase I clinical trials are conducted with malaria naïve adults, and such data may not have much relevance for children living in malaria endemic areas. Indeed, immunity to malaria seems to be related to age in an absolute manner. A study of migrant population that had moved from malaria non-endemic to malaria endemic region in Irian Jaya showed that the immunity was acquired faster by adults than children[89]. Subsequently, it has also been shown that this immunity increased during puberty and was predicted by levels of the pubertal steroid dehydroepiandrosterone sulfate[90,91]. Thus, even the whole sporozoite or the erythrocytic vaccines are likely to be effective only on naïve adults.

Conclusion

Vaccine development and field trials are lengthy and expensive. In most of the trials, including those in naïve volunteers, it is important to note that sterile immunity was not observed in a large proportion of the subjects for a significant length of time. This has important implications. Are children from endemic areas in a position to take frequent vaccination doses? The RTS,S as well as irradiated sporozoite vaccine might prove useful for transient visitors to endemic areas, such as tourists or military personnel. However, to manage the current burden of malaria in endemic areas, alternative methods such as insecticide spraying, insecticide-treated bednets, long-lasting insecticidal nets and combination drug therapies should be used to their maximum. For the community as a whole, perhaps it is time to introspect as to whether to invest heavily in vaccine trials, or to let them take a breather and stress on research towards a better understanding of the host-parasite interactions in the field.

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Annexure 1. Target antigens and candidate vaccines undergoing development

Parasite stage	Antigen	Location and processing	Candidate vaccine(s)	Stage of development	Reference
Pre-erythrocytic	CSP (Circumsporozoite protein)	Major surface protein of sporozoites, forms a dense coat on the parasite's surface; proteolytically cleaved on the sporozoite surface when parasites contact target cells	CSP fragments in various combinations and adjuvants (RTS,S; CS102; FP9-CS; ICC-1132.CS; PEV 302; PV-CSP; Ad35.CS; see Annexure 2)	Clinical trials	7*, 74, 96*
			Other CSP vaccines (DNA vaccines/live recombinant vaccines using MVA, FP9, Ad, Sindbis virus, yellow fever virus or attenuated influenza virus with or without prime boost)	Animal/Safety trials	7*
	LSA-1 (Liver stage antigen-1)	Parasitophorus vacuole, released in abundance during merozoite release	FMP-011 (*P. falciparum* LSA-1; see Annexure 2)	Clinical trials	74
	LSA-3 (Liver stage antigen-3)	Highly conserved protein expressed in both sporozoites and parasite liver-stages	PfLSA-3 (*P. falciparum* LSA-3; see Annexure 2)	Clinical trials	74
	TRAP (Thrombospondin related adhesion protein)	Sporozoite microneme transmembrane protein; appears to be released to parasite surface immediately before invasion	TRAP in various combinations and regimes (FP9 ME-TRAP/ FFM ME-TRAP; DDM-ME TRAP; see Annexure 2)	Clinical trials	93, 94
			PvTRAP (Syn representing *P. vivax* TRAP fragment containing the motif for sporozoites and hepatocytes interaction)	Mice, *Aotus* monkeys	95

Annexure 1 (contd.)

Parasite stage	Antigen	Location and processing	Candidate vaccine(s)	Stage of development	Reference
Asexual	AMA-1 (Apical membrane antigen-1)	Microneme type 1 integral membrane protein; proteolytically cleaved prior to relocalization to the merozoite outer membrane	AMA-1 (Various combinations and adjuvants: FMP2.1; AMA-1-C1; PEV301; PfAMA-1-FVO [25–545]; PfCP2.9; (see Annexure 2)	Clinical trails	7*, 74, 96*
	EBA-175 (*P. falciparum* erythrocyte binding antigen-175)	Released from micronemes at the time of schizont rupture	EBA-175 RII-NG (see Annexure 2)	Clinical trials	74
	DBL (*P. vivax* duffy-binding ligand)		PvRII (Rec conserved N-terminal RII region of *P. vivax* DBP)	*Aotus* monkeys	97
	GLURP (Glutamate-rich protein)	Paraitophorus vacuole, binds to merozoite surface	GLURP combinations (see Annexure 2)	Clinical trials	98, 74
	MSP-1 (Merozoite surface protein-1)	Merozoite surface, cleaved into four fragments during schizogeny, final cleavage during invasion	MSP-1 fragments in various combinations and adjuvants (FMP-1; $MSP\text{-}1_{42}$-FVO $MSP\text{-}1_{42}$-3D7; $MSP\text{-}1_{42}$-C1/ Alhydrogel), (see Annexure 2)	Clinical trials	7*, 74, 96*
			$MSP\text{-}1_{42}$+ $MSP\text{-}1_{19}$ (Rec *P. falciparum* MSP fragments)	Phase I; abandoned due to side effects	7*
			PvMSP-1 (Rec *P. vivax* $MSP\text{-}1_{14}$ + Rec $MSP\text{-}1_{20}$)	*Aotus* monkeys	99
			Pv200L (Rec *P. vivax* MSP-1 fragment with sequence homology to *Pf*190L)	*Aotus* monkeys	100
	MSP-2 (Merozoite surface protein-2)	Merozoite surface	MSP2-C1/ISA720 (*P. falciparum* MSP-2 conserved region, see Annexure 2)	Clinical trials	74
	MSP-3 (Merozoite surface protein-3)	Merozoite surface	MSP-3 LSP (see Annexure 2)	Clinical trials	101
	PfEMP-1 (Erythrocyte membrane protein-1)	*Plasmodium falciparum* infected erythrocyte surface	PfEMP1-CIDR1α (Rec cysteine-rich interdomain region 1α)	*Aotus* monkeys; failed to protect	102
			PfEMP1-NTS-DBL-1α/x (Rec NTS-DBL-1α/x domains from 3D7 strain) in Montanide ISA 720	Rats	103
	Pfen (enolase)	Merozoite surface, in addition to cytoplasm	Rec *P. falciparum* enolase (aa 1–446)	Mice	61

Annexure 1 (contd.)

Parasite stage	Antigen	Location and processing	Candidate vaccine(s)	Stage of development	Reference
	PfP0 (ribosomal phosphoprotein P0)	Merozoite surface, in addition to ribosomes	Rec *P. falciparum* PfP0 N-terminus (aa 1–61) and C-terminus (aa 62–316)	Mice	104
	RESA (Ring-infected erythrocyte surface antigen)	Microneme	Combination B (see Annexure 2)	Clinical trials	105
	SERA-5 (Serine repeat antigen)	Accumulates in the parasitophorous vacuole of trophozoites and schizonts; processed into 3 fragments and a complex consisting of an N-terminal 47 kDa and C-terminal 18 kDa product which associates with the surface of merozoites	FB-23 (see Annexure 2)	Clinical trials	7*, 96*
Sexual	Pfs25/Pvs25	Expressed on zygotes and mature ookinete stages of parasites expressed in the mosquito midgut and not in the vertebrate host	Pvs25H (see Annexure 2)	Clinical trials	106
			Pfs25 (Rec 23 -193 fragment of *P. falciparum* Pfs25)	TBA *in vitro*	107
			Rec Pvs25H in Montanide ISA 720 or Alhydrogel	TBA *in vitro*	108
	Pvs28	Expressed on the surface of zygotes and ookinetes expressed in the mosquito	Rec Pvs28, DNA in different prime boost strategies	TBA *in vitro*	109
Others	Pfs230	Gamete/zygote/ookinete surface molecules expressed by the parasite as it matures in the human host	r230/MBP.C (Rec Pfs230 region C), DNA vaccine	TBA *in vitro*	110
Multistage	GPI (Glycosylpho-sphatidylinositol)	Tethers several of *Plasmodium* molecules to membrane	Syn *P. falciparum* GPI	Mice	69
	—	—	Various combinations (DNA-MuStDO5; L3SEPTL; NMRC-M3V-Ad-PfCA; SPf66; NYVAC-Pf7; see Annexure 2)	Abandoned or in clinical trials	7*, 74

MVA–Modified Ankara virus; FP9–Fowl pox virus 9; Ad–Adeno virus; Rec–Recombinant protein; Syn–Synthetic peptide; aa–Amino acid; TBA–Transmission blocking activity; *Reviews cited.

Annexure 2. Major candidate vaccines used in clinical trials

Parasite stage	Candidate vaccine	Antigen and adjuvant	Stage of development	Reported results (reference)
Pre-erythrocytic CSP	RTS,S	Rec. fusion product of *P. falciparum* CSP aa 207–395, central NANP repeat sequence, and T-cell epitopes fused to HBsAg in AS02A, AS02B, or AS02D (combination of Monophoshphoryl Lipid A and saponin derived QS2)	Phase III completed	Prevented first malaria attack in 1–4 years olds by about 30%; overall incidence of severe disease was decreased 58% over a 6-month follow-up period (7*, 92*)
	CS102	Syn 102-amino acid C-terminus of *P. falciparum* CSP, with Montanide ISA 720	Phase IIa completed	Elicited both antibody and cellular immune response in humans; failed to show protection against malarial challenge (7*)
	FP9-CS	FP9 expressing the full-length *P. falciparum* CSP + MVA expressing CSP	Phase IIa completed	Induced modest immune responses in malaria naïve adults, but showed no evidence of efficacy in a sporozoite challenge (111)
	ICC-1132.CS	Rec HBcAg expressing 1 B-cell (NANP tandem repeat sequences) and 2 T-cell epitopes of *P. falciparum* CSP in Montanide ISA 720 or Al(OH)3	Phase IIa completed	No evidence of protection from experimental challenge with sporozoites in malaria naïve adults; approach discontinued (112)
	PEV302	Syn UK-39, cyclic peptide of 5 NANP repeats from *P. falciparum* CSP coupled to IRIV	Phase Ia completed	Induced a long-lived parasite-inhibitory antibody response in malaria naïve adults (113)
			Phase Ib trials started in Tanzania	Data not yet available
	PV-CSP	Syn *P. vivax* CSP peptides — N-terminus, repeat region & C-terminus — in Montanide ISA 720	Phase Ia completed	Induced specific antibody response; IFN-γ production by T-cells induced in 94% malaria naïve participants (114)
	Ad35.CS	Rec Ad expressing consensus *P. falciparum* CSP N-terminus with Al(PO4) (115)	Phase I trial underway	Data not yet available
LSA-1	FMP-011	Rec protein containing the N-terminus, two 17 aa repeat units, and C-terminus of *P. falciparum* LSA-1 in various adjuvants (116)	Phase I/IIa trials launched in USA	Results not yet published
LSA-3	PfLSA-3-Rec	3 Rec peptides covering major part LSA-3 *P. falciparum* K1 strain	Phase I and IIa launched in Netherlands	Data not yet available
TRAP	FP9 ME-TRAP/	FP9/MVA expressing *P. falciparum* TRAP, joined to a ME	Phase IIb completed	Ineffective at reducing the natural infection rate in semi-immune

Annexure 2 (contd.)

Parasite stage	Candidate vaccine	Antigen and adjuvant	Stage of development	Reported results (reference)
	FFM ME-TRAP	(Multiple Epitope string: 14 MHC class I, 3 class II, and 2 B-cell epitopes form 6 pre-erythrocytic antigens, and a *P. berghei* epitope for stability testing)		African adults or children; induction and persistence of IFN-γ responses suppressed by *Plasmodium falciparum* infection (93, 117)
	DDM-ME TRAP	DNA priming + MVA encoding ME-TRAP	Phase I completed	Delay to parasitaemia in malaria naïve adults, no significant efficacy against parasitemia in semi-immune adults (77, 94)
Asexual AMA-1	FMP2.1	Rec 83-531 aa of the *P. falciparum* 3D7 AMA-1 in AS02A or AS02B	Phase Ib completed	Vaccine elicited potent humoral and Th1-biased cellular immune responses in malaria naïve adults (18)
			Phase I pediatric and Phase II trial started in Mali	Data not yet available
	AMA1-C1	Rec AMA1 derived from the FVO and 3D7 clones of *P. falciparum* in Alhydrogel	Phase I completed	Induces a significant humoral immune response in malaria-exposed individuals; booster administration a year later did not result in increased antibody levels. Increased antibody levels not associated with *in vitro* growth inhibition (119)
		AMA1-C1 in CPG 7909	Phase I trial underway in USA	Data not yet available
	PEV301	Syn AMA49-C1, a 49-aa cyclic peptide of domain III of *P. falciparum* AMA-1 coupled to IRIVs	Phase Ia completed	Induced a long-lived parasite-inhibitory antibody response in malaria-naïve adults (113)
			Phase Ib trials started in Tanzania	Data not yet available
	PfAMA-1-FVO [25-545]	Rec *P. falciparum* AMA-1 (25-545) in Al(OH)3/Montanide ISA 720/ASO2	Phase Ib study underway in Mali	Data not yet available
	PfCP2.9	Rec chimeric protein: domain III of AMA-1 (*P. falciparum* 3D7) + $MSP\text{-}1_{19}$ (*P. falciparum* Wellcome/K1) in Montanide ISA 720 (120)	Phase I trial completed in China	Results not yet published
EBA-175	EBA-175 RII-NG	Syn *P. falciparum* EBA Region II-nonglycosylated in Al(PO4)	Phase 1 trial underway in USA	Data not yet available

Annexure 2 (contd.)

Parasite stage	Candidate vaccine	Antigen and adjuvant	Stage of development	Reported results (reference)
GLURP	GLURP	*P. falciparum* LSP $GLURP_{85–213}$ in Montanide ISA 720 or Al(OH)3	Phase I completed	Vaccine induced dose-dependent cellular and humoral immune responses, with high levels of IgG1antibodies in malaria naïve adults (98)
	GMZ2	Rec *P. falciparum* hybrid protein with GLURP N-terminus and MSP-3 C-terminus) in various adjuvants	Phase Ia trial completed in Germany	Data not yet available
			Phase Ib trial underway in Gabon	(74)
MSP-1	FMP1/AS02A	Rec $MSP\text{-}1_{42}$ of *P. falciparum* 3D7 in AS02A	Phase Ib completed	A statistically significant antibody response to a 3-dose regimen of FMP1/AS02A in a population with substantial baseline antibody was observed (121)
			Phase IIb completed in Kenyan children	Showed no efficacy to reduce clinical episode of malaria [unpublished data (96*)]
	$MSP\text{-}1_{42}$-FVO/ $MSP\text{-}1_{42}$-3D7	Rec $MSP\text{-}1_{42}$ derived from the FVO or 3D7 parasite lines of *P. falciparum* in Alhydrogel	Phase I completed	Not sufficiently immunogenic to generate a biologic effect in *in vitro* growth inhibition tests (122)
	$MSP\text{-}1_{42}$-C1-Alhydrogel	*P. falciparum* $MSP\text{-}1_{42}$ in multiprotein complex in Alhydrogel, with or without CPG 7909	Phase I trial underway in USA	Data not yet available
MSP-2	MSP-2-C1/ ISA720	*P. falciparum* MSP-2 conserved region in Montanide ISA720	Phase I trial underway in Japan	Data not yet available (96*)
MSP-3	MSP-3 LSP	LSP 181 to 276 of MSP-3 of *P. falciparum* strain Fc27, in Montanide ISA 720 or Al(OH)3	Phase Ib completed	Stimulated an enhanced cell-mediated immune response in adults living in a malaria endemic area (101)
			Pediatric testing to start in Tanzania and Burkina Faso	(74)
RESA	Combination B	Rec protein comprising of relatively conserved blocks 3 & 4 of MSP-1 fused with a universal T-cell epitope of CSP of *P. falciparum* + near full-length MSP-2 (3D7) + C-terminal 70% of RESA (FCQ-27/PNG) in Montanide ISA720	Phase II completed	62% reduction in parasite density in vaccinees; breakthrough parasites showed a significant increase in FC27 allele genotype, the opposite dimorphic form of MSP-2. A new version using both variants of MSP-2 is being developed (7*, 96*)

Annexure 2 (contd.)

Parasite stage	Candidate vaccine	Antigen and adjuvant	Stage of development	Reported results (reference)
SERA-5	FB-23	Syn *P. falciparum* SERA-5 (aa 57–94)	Clincal trial underway in Japan	Data not yet available (7*,96*)
Sexual Pvs25	Pvs25H	Rec Pvs25 (aa 23 to 195) of *P. vivax* Salvador 1 isolate in allhydrogel	Phase I clinical trial completed	Generated transmission blocking antibodies in malaria naïve adults (106)
Multistage	DNA-MuStDO 5 (Multi-stage DNA vaccine operation 5)	6 *P. falciparum* antigens — CSP, Sporozoite surface protein 2, Exported Protein 1, C-terminus of LSA-1, LSA-3, expressed in the VCL-2510 DNA vector, with GM-CSF DNA as adjuvant	Phase IIa completed	No evidence of protection obtained in Phase IIa challenge trials (7*)
	L3SEPTL	*P. falciparum* LSA-1, LSA-3, EXP-1(Exported protein-1), Pfs16, STARP, TRAP expressed in FP9 or MVA vectors with or without prime boost regimen	Phase IIa trials completed	Results not yet published
	NMRC-M3V-Ad-PfCA	*P. falciparum* CSP and AMA-1 in adenovirus	Phase I/IIa trials underway in USA	Results not yet published
	SPf66	*P. falciparum* merozoite protein-derived 3 Syn peptides + PNANP sporozoite repeat sequence in Al(OH)3	Abandoned after phase III trials	Too low an efficacy (6*)
		SPf66 in QS21	Phase I trial completed	Increased antibody titers in malaria naïve adults (123)
	NYVAC-Pf7	Poxvirus-vectored *P. falciparum* multiantigens —CSP, TRAP, LSA-1, AMA, MSP-1, SERA, Pfs25	Phase I/IIa completed; Not progressing	Variably immunogenic, failed to protect malaria naïve volunteers (124, 125*)

MVA– Modified Ankara virus; FP9–Fowl pox virus 9; HBcAg–Hepatitis B core antigen; HBsAg–Hepatitis B antigen; IRIV–Immunopotentiating reconstituted influenza virosomes; Ad– Adeno virus; Rec–Recombinant protein; Syn– Synthetic peptide; aa–Amino acid; *Reviews cited.

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