Review

Development in malarial vaccine: A review

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ABSTRACT: Malaria, a vector-borne infectious disease, is currently a grave and universal concern with a significant social, economic, and human cost, mainly in developing countries. In addition, the emergence and spread of resistance to antimalarial therapies have further aggravated the global situation. Currently most of the research is focused on development of antimalarial drugs, drug resistance, and novel formulations to maximize the therapeutic effect. A number of novel molecules potentially active against malarial parasites are being developed. A vaccine is still viewed as a critical part of a long-term malaria control strategy. In the last several years various studies have shown significant progress in the development of vaccines against malaria. Advancement in vaccine technology and immunology is being used to develop malaria subunit vaccines that would open up new vistas for effective treatment and control of malaria. The development of an effective malaria vaccine represents one of the most important approaches that would provide a cost-effective intervention in addition to currently available malaria control strategies. An overview on progress in antimalarial vaccines is presented.

Keywords: Malaria, plasmodium, vaccines, adjuvant

1. Introduction

Malaria is a debilitating parasitic disease. According to the Roll Back Malaria (RBM) Partnership, each year approximately 860,000 (89% were in the African Region, followed by 6% in the Eastern Mediterranean and 5% in the South-East Asia Regions) lives, mainly

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children and women, succumb to it (*1,2*).

Most of the drugs used in antimalarial chemotherapy are particularly active against the asexual blood forms of the parasite, which are responsible for the clinical symptoms of the disease (*3*). The increasing resistance of the malaria parasite *Plasmodium falciparum* to currently available drugs and especially to chloroquine necessitates a continuous effort to develop more effective therapeutic options (*4,5*).

Malaria continues to exact a devastating social and economic cost across the globe. It strikes hardest at some of the poorest nations and is a significant root and outcome of poverty. This is endemic in many developing nations; additionally malaria is also of serious concern to travelers, especially in the absence of a vaccine and in the face of widespread resistance to several antimalarial drugs (*6*). The most common etiological agent of malaria is *P. falciparum*, a highly evolved unicellular parasite whose life cycle, shared between an Anopheles mosquito vector and the human host, exhibits striking biological features that have been exploited to seek intervention strategies for malaria therapy (*7*). A logical and rational approach for vaccine development is imperatively needed. Devising an effective malaria vaccine would certainly help in limiting unacceptably high morbidity (*8*). Due to the complexity of the malarial parasite's life cycle, a better understanding of its molecular interactions in invasion is required (*9,10*).

Because of the rapid emergence of drug resistance and unclear mechanisms, much money has been wasted in many malaria endemic sites. Therefore a vaccine seems to be an alternative and pragmatic approach to eradicate the disease. Even a modestly efficacious malaria vaccine may protect hundreds of thousands of people from disease and death each year. The development of an effective malaria vaccine represents one of the most important approaches that would provide a cost-effective intervention in addition to currently available malaria control strategies. A number of antigens (a dozen or so) derived from different stages of the parasite's life cycle have been described and are in preclinical or clinical vaccine trials (*11*).

The recent sequencing of genomes of the *Plasmodium* species causing malaria offers immense opportunities to aid in the development of new

therapeutics and vaccine candidates through Bio-informatics tools and resources. One such database that can significantly promote the vaccine research is the MalVac Database (*12*). Recent launching of a massive vaccine trial spearheaded by British drugmaker GlaxoSmithKline PLC in collaboration with the PATH (Programs for Appropriate Technologies in Health) Malaria Vaccine Initiative has been reported. This initiative might accelerate development of a new generation of malaria vaccines (*http://www.malariavaccine.org/news-press-kits-09252008.php*).

An ideal vaccine should be safe with no or minimal side-effects, easy and cheap to manufacture, stable for storage/transport, easy to administer, could be given to infants (ideally alongside other childhood vaccinations), and should stimulate life-long protection against all forms of the disease. Although much effort is currently directed at disease treatment through the development of new antimalarial drugs, vaccines enjoy many intrinsic advantages. The desirable frequency of vaccine treatment is perhaps in the range of once or twice per lifetime, or at least, once or twice per year. Vaccines are relatively cheap to produce, if albeit not necessarily easy to discover, making them of special interest to developing countries. The ongoing spread of drug-resistant forms of malaria in combination with the side-effects associated with prevention and treatment of this disease have provided impetus for the development of a safe and effective vaccine.

2. Progress in the development of antimalarial vaccines

It is important to understand the pathogenesis of the disease, including the life cycle (Figure 1) of the parasite and the in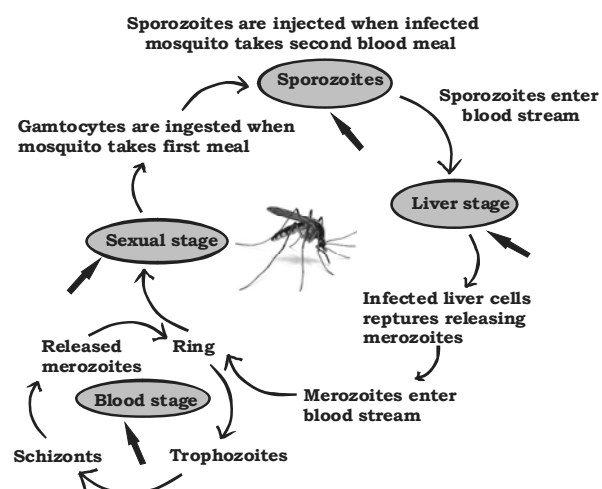teraction between the host immune response and the parasite, for better preventive and therapeutic modalities against malaria.

The initial stage of infection involves the inoculation of sporozoites into the blood stream known as the pre-erythrocytic stage. This is an asymptomatic phase associated with an initial humoral response. The phase is followed by multiplication and infection of hepatocytes (*13*).

During this phase, a cell-mediated CTL (cytotoxic T lymphocyte) response is elicited. Last comes the erythrocytic phase, where infected hepatocytes rupture, releasing thousands of daughter merozoites back into the blood, where they invade circulating erythrocytes and which is associated with the clinical symptoms of the disease and correlated with both humoral and cellular responses (Figure 1) (*14*).

Vaccines against malaria have been designed to work at these different stages of the life cycle of the parasite (Table 1). Apart from the life cycle one can consider DNA and T-Cell targeting vaccines. Recently developed transmission-blocking vaccines are of considerable importance (*15*). In addition, potentially important targets for naturally acquired immunity have been exploited. The variant surface antigens (VSA) families present on the surface of infected erythrocytes particularly the PfEMP1 is well characterized. A VSA-based vaccine may prolong the protection level by preventing the parasites from expressing the VSA and will render the disease less severe (*16*).

Although promising an individual antibody response may vary between each domain as PfEMP1 exhibits a domain structure. Recent studies simultaneously observed that potential cross-reactivity in response to one of the domains- (Duffy-binding like domain) DBL4γ suggest reconsidering the vaccine for further improvement in triggering an invariable antibody response (*17*).

On the other hand a carbohydrate-based synthetic vaccine is also being developed, which will be discussed in a later part of this review. Based upon the above mentioned targets there are two standard models: (*i*) Vaccines to prevent clinical disease using irradiated sporozoites (*18*) and (*ii*) Vaccines to prevent mortality based on innate immunity (*19*).

Development of an effective malaria vaccine is a daunting task as the parasite undergoes changes in its morphological forms and surface antigens. These features enable the parasite to evade protective immune responses and vaccines based on protective immunity and are unlikely to effectively curtail transmission. As a result, the acquisition of long-term sterilizing immunity characterized by recovery from many infectious diseases does not occur with malaria. Hence vaccines inducing artificially acquired active immunity are the need of the hour and the recombinant pre-erythrocytic vaccine RTS, S might hold promise for the near future.

Figure 1. Malaria vaccines targeting life cycles: Targets-sporozoite, liver stage, blood stage, sexual stage. (*www.iavireport.org/Issues/1102/malariavax.asp; http://encarta.msn.com/media_461541582/life_cycle_of_the_malaria_parasite.html*)

Table 1. Possible targets and immune mechanism of antimalarial vaccines

S. No.	Targets	Immune mechanism
1	Pre-erythrocytic Stage	
	Sporozoites	Antibodies
	Liver forms	CD4+ and CD8+ T-Cells/antibodies
2	Asexual blood Stage	
	Erythrocytes (infected)	Antibody-includes inhibition of RBC invasion
3	Sexual Stage	
	Gametocytes	Antibody-blocking of activity (pre- and post-fertilization in the mosquito)

Table 2. Summary of antimalarial vaccines

S. No.	Vaccines	Type	Antigen	Mechanism	Site of Action
1.	RTS, S	Pre-erythrocytic	Fusion of C-terminal regions of CSP with the hepatitis B surface antigen	Prevents sporozoites from invading hepatocytes; Eliminating infected hepatocytes	Sporozoite
2.	FFM ME-TRAP	Pre-erythrocytic	Two attenuated POX virus vectors	Both deliver the pre-erythrocytic malaria antigen	Sporozoite
3.	ICC-1132	Pre-erythrocytic	Modified hepatitis B virus core particle (HBc) bearing putative protective T- and B-cell epitopes	Generate a potent anti-CSP antibody response	Sporozoite
4.	Liver Stage Antigen	Pre-erythrocytic	Liver stage antigens-1 and 3 (LSA-1 and LSA-3)	Shows antigenic effect on liver-stage parasites; Expressed solely in infected hepatocytes	Acts in liver schizogony and merozoite release
5.	MSP (Merozoites Surface Protein)	Blood Stage	Merozoite surface protein	Hinders Interactions between merozoites and erythrocytes during initial contact	Merozoite/Erythrocytes
6.	AMA1 (Apical Membrane Protein)	Blood Stage	Apical membrane antigen-1	Ceases the invasion of host cell immune responses by *Plasmodium* profound parasite-inhibitory effects	Erythrocytes
7.	EBA-175 RII-NG	Blood Stage	Erythrocyte binding antigen (EBA-175)	Blocks incursion of host cells and an intracellular translocation of machinery by parasite	Erythrocytes
8.	PfEMP1	Blood Stage	Erythrocyte Membrane Binding antigen	Immunity to the surface of the trophozoite-infected RBC	Erythrocytes
9.	PfCP2.9	Combination blood stage	AMA1/MSP1 chimeric recombinant antigen	Hinders Interactions between merozoites and erythrocytes during initial contact	Erythrocytes
10.	AMA1-C1/Alhydrogel® plus CPG7909	Asexual Blood-Stage Vaccine	Apical membrane antigen-1	Profound parasite-inhibitory effects. This is the first reported use in humans of an investigational vaccine	Erythrocytes
11.	MSP3/GLURP (GMZ2) AlOH	Combination blood stage	Recombinant GLURP-MSP3 fusion antigen	GLURP and MSP3 antigenicity	Erythrocytes
12.	Pfs230	Sexual stage	Gamete-specific surface antigen	Block the infectivity of gametes	Gametocytes
13.	Pfs25 and Pvs25	Transmission blocking vaccine	Surface antigen of mosquito stage of the malaria parasites	Kills the mosquito stage of parasite	Mosquito stage of parasite
14.	FFM ME-TRAP + PEV3A	Combination Multi-stage Vaccines	Targeting different stages of the malaria life cycle		
15.	NYVAC-Pf7	Single NYVAC genome containing genes encoding seven *Plasmodium falciparum* antigens			
16.	Synthetic GPI vaccine	Endotoxin of parasitic origin	Prevent the pathology and fatalities of severe malaria		

2.1. *Pre-erythrocytic vaccines*

A pre-erythrocytic vaccine is designed to target sporozoites or schizont-infected liver cells and thus prevent the release of primary merozoites from infected hepatocytes. Sporozoites constitute the infective stage of the malarial parasite and they are ideally the target for a malaria vaccine. Vaccines against the pre-erythrocytic stages of malaria hold the greatest promise as an effective intervention tool against malaria. The

pre-erythrocytic stage vaccine (PEV) includes antigens from the sporozoites and liver stages. The PEV is unique in that it can induce a state of strong, sterile immunity both in humans and in animal models (*20*).

The ideal vaccine for this stage would induce high titers of functional antibodies against sporozoites to prevent all parasites from entering the liver stage, and induce potent cytotoxic T-lymphocyte immunogenicity against the liver stage to kill infected hepatocytes, while not harming the human host. The lead candidate vaccine of this type is RTS, S, a recombinant protein vaccine. It targets the sporozoite stage preventing the invasion of hepatocytes by sporozoites or destroys parasites in infected hepatocytes and thus prevents both clinical disease and the transmission of malaria (Figure 2) (*21-23*).

2.1.1. *Circumsporozoite protein (CSP) based vaccines*

CSP expressed on the surface of developing and mature sporozoites are considered to be an important target of antibody and T cell-mediated responses to sporozoites (*24*). Due to high immunogenicity and a crucial role in hepatic cell invasion by CSP this molecule has been considered as an excellent target for antimalarial vaccine development. CSP based vaccine is classified into recombinant and synthetic (*25*). The CSP displays a characteristic common molecular structure in all *Plasmodia*, consisting of a central tandem amino acid repeat region and two relatively conserved domains named regions I and II (containing great genetic variability in some residues), localized at the protein's amino- and carboxy-terminal ends, respectively (Figure 3) (*26,27*). PEV has been primarily based on the CSP. In particular, the central repeat region that contains an immunodominant B cell epitope represented the target of the initial vaccine trials (*28-30*).

(*a*) *RTS, S* – RTS, S is a recombinant pre-erythrocytic vaccine consisting of the circumsporozoite protein found on the surface of the sporozoite stage of *P. falciparum*. It's a fusion of the central repeat and C-terminal flanking regions of CSP with the hepatitis B surface antigen (HBsAg), combined and named RTS, and formulated with the AS02A Adjuvant System (*21*). This antigen elicits antibodies that are capable of preventing sporozoites from invading hepatocytes, and a cellular response that is capable of eliminating infected hepatocytes (*31,32*). Different types of RTS, S vaccines depending upon adjuvant are: RTS, S/AS02A, RTS, S/AS02B, RTS, S/AS02D, RTS, S/AS02E. The most successful malaria vaccine to date, the recombinant protein RTS, S administered with the adjuvant AS02A, has shown protection against experimental and natural *P. falciparum* sporozoite challenge in humans (*33,34*).

A trial in southern Mozambique aimed to assess the safety, immunogenicity, and initial efficacy of the malaria vaccine RTS, S formulated in the AS02 adjuvant
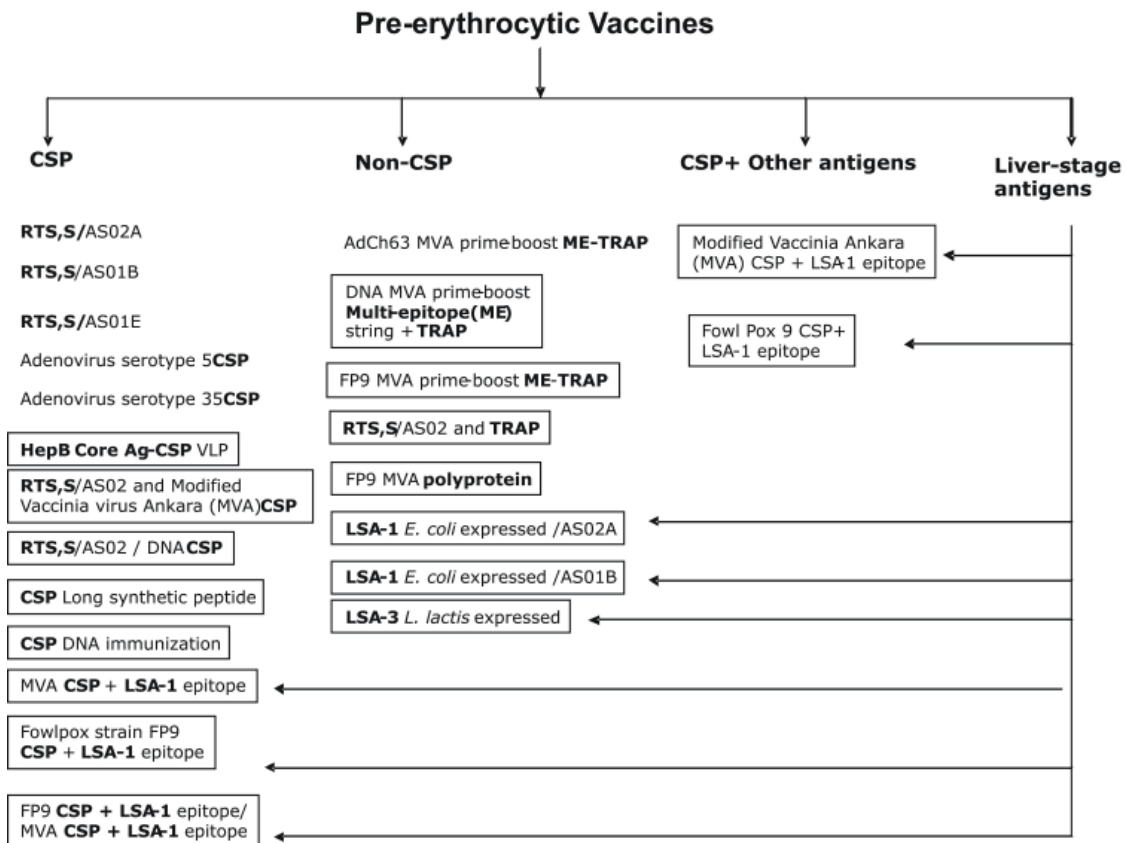

Figure 2. Pre-erythrocytic vaccines in various phases of clinical trials. Vaccines in BOX are withdrawn from clinical trials. (*http://www.who.int/vaccine_research/documents/Malaria%20Vaccine%20Rainbow%20Table_Clinical_Oct_2008.pdf*)
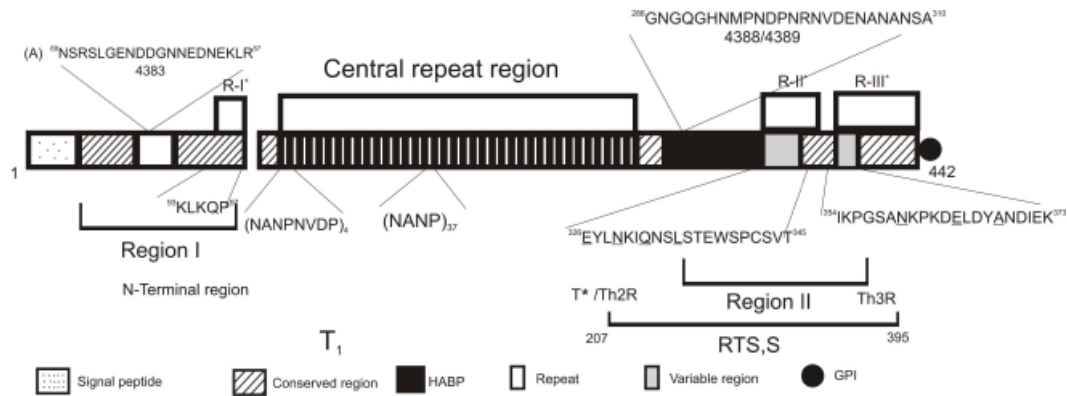
Figure 3. Various regions of CSP vaccine.

system, AS02D (with the letter D indicating the pediatric formulation) has proved that the vaccine is having a good safety profile and is protective in those most vulnerable to the disease-infants less than 1 year old (*35*). This trial was followed by recent Phase IIb studies of the vaccine in Bagamoyo, Tanzania. Although the vaccine was co-administered to infants with other routinely delivered EPI immunizations (the World Health Organization's Expanded Program on Immunization), it did not interfere with the immunogenicity of the multiple co-administered antigens. A 65% rate of protection against new infections was observed (*36*). To confirm the efficacy of RTS, S, a study sponsored by the PATH Malaria Vaccine Initiative (MVI) and GlaxoSmithKline Biologicals found that this particular malaria vaccine has significant efficacy with fewer serious adverse effects in clinical malaria in children 5 to 17 months of age (*37*).

A large scale study (Phase III) of RTS, S/AS01E (Mosquirix®) vaccine developed by GSK (Glaxo Smithkline Biologicals) has been launched and is expected to be undertaken at 11 different sites in seven countries in Sub-Saharan Africa involving 16,000 children and infants (*38*).

(*b*) *FFM ME-TRAP* – FFM ME-TRAP denotes two recombinant viral vectors, attenuated fowl pox strain FP9 (FP9 ME-TRAP) and modified Vaccinia Virus Ankara (MVA ME-TRAP). Both encode the pre-erythrocytic malaria antigen construct thrombospondin-related adhesion protein (TRAP) coupled to the multiple epitope (ME) string (*39*). Outcome from a Phase IIb trial with vaccination regimen FFM ME-TRAP indicated that the vaccine showed weak immune response and was not protective against episodes of clinical malaria among children in a malaria endemic area (*40*).

(*c*) *ICC-1132* – ICC-1132 is a malaria vaccine candidate based on a modified hepatitis B virus core particle (HBc) bearing putative protective T- and B-cell epitopes from the repeat region and a universal T-cell epitope from the C terminus of CSP of *P. falciparum* (Figure 4). It was used to generate a potent anti-CSP antibody response. This vaccine has been found to be highly immunogenic in mice and in *cynomolgus*
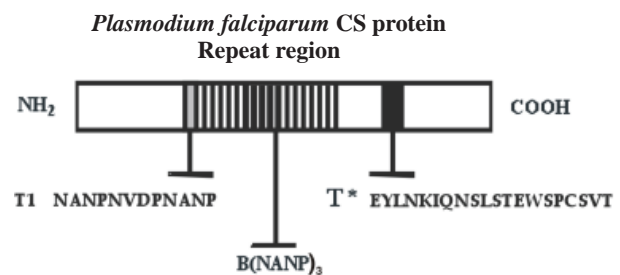

Figure 4. *Plasmodium falciparum* **CS proteins [B-cell epitope; (NANP)₃; T1-epitope; T*-epitope]** (*41,42*).

monkeys. ICC-1132 elicits potent antibody responses in mice primed with *P. falciparum* sporozoites. This suggests a potential advantage of enhancing the sporozoite-primed responses of semi-immune individuals in endemic areas (*41,42*).

In a recent trial of ICC-1132 (Malariavax), a novel CS based malaria vaccine was found to be safe and well tolerated but malaria specific antibody responses were relatively weak as compared to the responses to the immunogen (ICC-1132) and to HBc. When adjuvanted with alum (Alhydrogel® formulation), the vaccine was poorly immunogenic suggesting the use of a more powerful adjuvant system (*43*).

(*d*) *CSP (Long synthetic peptide)* – One of the major targets of antibody and T cell-mediated responses to sporozoites is the CSP expressed on the surface (*54*). CSP derived peptides are presented in association with MHC molecules on the surface of infected liver cells as evidenced by CSP-specific T cell recognition (*44*).

(*e*) *CSP + other antigens* – Modified Vaccinia Ankara (MVA) CSP + LSA-1 epitope, and Fowl Pox 9 CSP + LSA-1 epitope are two types of vaccine which have CSP along with some other antigens like LSA-1. Unfortunately both of them have failed in their Phase Ia clinical trials. Apart from these CSP vaccines, numerous CSPs became obsolete in various stages of their clinical trials. Some of them are: HepB Core Ag-CSP VLP, RTS, S/AS02 and modified vaccinia virus Ankara (MVA), RTS, S/AS02/DNA CSP (NMRC-GSK), CSP Long synthetic peptide, CSP DNA immunization, MVA CSP + LSA-1 epitope,

Fowl pox strain FP9 CSP + LSA-1 epitope, and FP9 CSP + LSA-1 epitope/MVA CSP + LSA-1 epitope.

2.1.2. *Liver-stage antigens (LSAs)*

The liver plays a crucial role in the *Plasmodium* life cycle, because hepatocytes are an obligatory site for schizogony, a process of amplification and molecular changes for the parasite. In *P. falciparum*, at least two of the relevant antigens (LSA-1 and LSA-3), have been identified and characterized (*45-47*). LSAs 1 and 3 expressed by liver-stage parasites and both pre-erythrocytic and a blood-stage parasites respectively showed promising antigenic and immunogenic properties. LSA-1, from current evidence, is one of the few antigens exclusively expressed in hepatocytes (*48,49*). Immunization studies with various LSA-3 antigens on chimpanzees (*Pan troglodytes*), the primates most closely related to humans, induced protection against successive challenges with large numbers of *P. falciparum* sporozoites. These surface proteins are expressed solely in infected hepatocytes and thought to have a role in liver schizogony and merozoite release (*50*). The different stages of the parasite express different antigens. A vaccine effective in killing liver-stage parasites may not inhibit the growth of blood-stage parasites and the above study did not confirm whether it was liver-stage or blood-stage protection (*51,52*). Although the exact function of LSA-1 for the parasite remains unknown, there is still evidence that this antigen is an attractive target for vaccine design at both the T-cell and B-cell level.

2.2. *Blood-stage/erythrocytic vaccines*

For an asexual stage malaria vaccine, the impetus came from the establishment of parasite culture by Trager and Jensen (*53*) and peptide biology. Blood stage vaccines (Figure 5) aim to impact the disease causing stage of the parasite's life cycle, and research in this area has focused mainly on AMA1 and MSP1 (*54*) antigens that are used by the parasite to invade the human red blood cell and cause disease. There are two possible classes of blood-stage vaccine: anti-invasion and anti-complication. A vaccine that could prevent invasion of red blood cells by merozoites would prevent malaria disease. Blood-stage vaccines reduce morbidity and mortality due to severe malaria, and are not intended to prevent infection (*55*). Development of such vaccines has been hampered by the lack of an established human challenge model, by the limitations of available animal models, and by unclear immunological correlates of protection (*56*). Despite these challenges, several antigens expressed by merozoites, the extra cellular form of the parasite that infects erythrocytes, have emerged as promising vaccine candidates. Druilhe and colleagues put forward encouraging findings from a Phase I trial of merozoite surface protein (MSP) (*57*).

2.2.1. *MSP (merozoite surface protein)*

During initial contact MSPs, often referred as "surface proteins", mediate the interactions between merozoites and erythrocytes (*58,59*). MSPs are situated on the merozoite surface, and are associated with merozoite surface molecules. In *P. falciparum*, this protein ranges in size from 185 to 200 kDa and is attached at its C terminus to the parasite plasma membrane *via* a glyco-sylphosphatidylinositol anchor (*60*). These proteins are of great importance in developing potential targets as candidates for an anti-malarial vaccine (*61,62*).
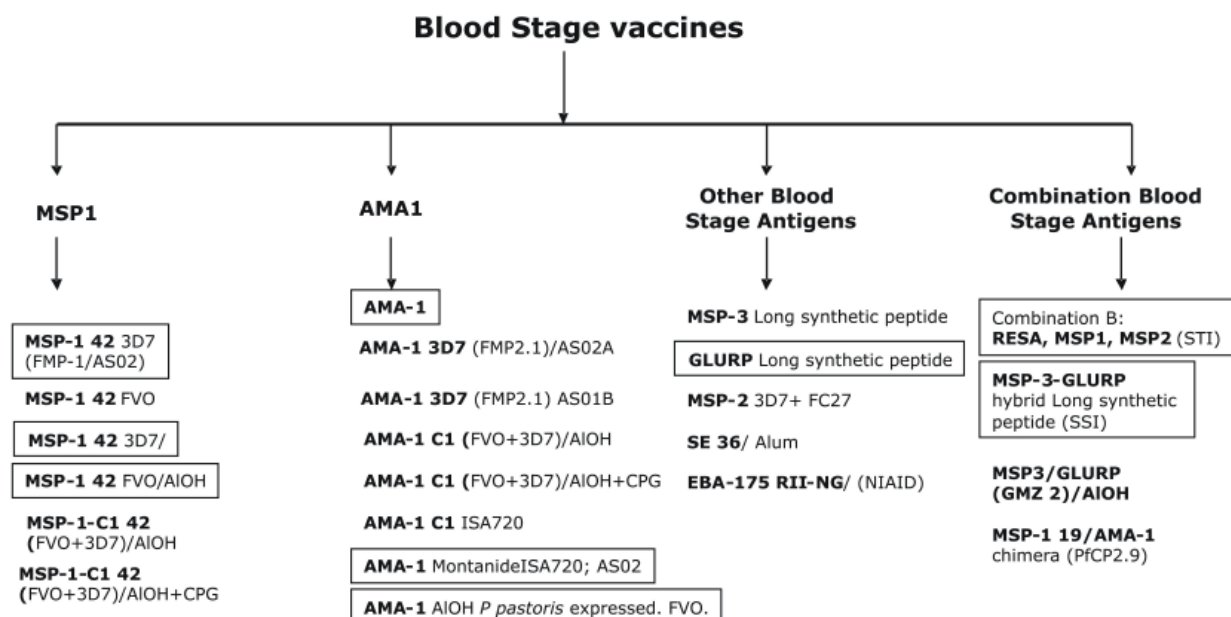

Figure 5. Blood stage vaccines in various phases of clinical trials. Vaccines in BOX are withdrawn from clinical trials. (*http://www.who.int*)

The major MSP-1 is one of the most widely studied parasite antigens from the erythrocyte stage of infection by *P. falciparum* (*63,64*). It is secreted as a 195 kDa protein that is proteolytically cleaved (Figure 6) to form four fragments, of 83 kDa, 30 kDa, 38 kDa, and 42 kDa, during merozoite maturation (*65-67*). The 42 kDa C-terminal fragment (MSP-142) is further processed to 33 kDa and 19 kDa. Only the MSP1-19 fragment remains on the merozoite surface at the time of erythrocyte invasion. It contains two epidermal growth factor-like modules that are anchored to the surface *via* a glycosylphosphatidylinositol moiety. Both MSP-119 and MSP-142 are leading vaccine candidates for the blood stage of *P. falciparum* (*68*).

2.2.2. *Apical membrane antigen (AMA1)*

AMA1 is a micronemal protein of apicomplexan parasites that appears to be essential during the invasion of host cell immune responses by *Plasmodium*. AMA1 can have profound parasite-inhibitory effects; both have been measured *in vitro* and in animal challenge models, suggesting AMA1 as a potential vaccine component (*69,70*). AMA1 is a structurally conserved type I integral membrane protein varying between 556 to 563 amino acids and is an apical membrane antigen 1 of the malarial parasite *P. falciparum* (PfAMA1). It is a merozoite antigen that is considered a strong candidate for inclusion in a malaria vaccine (Figure 7) (*71*).

2.2.3. *AMA1-C1/Alhydrogel® plus CpG 7909*

The AMA1-Combination 1 vaccine was developed by the Malaria Vaccine Development Branch of the National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health, USA. It contains an equal mixture of yeast expressed recombinant allelic proteins based on sequences from the FVO (fragment from the Vietnam-Oak Knoll) and 3D7 strains of *P. falciparum*, adsorbed on Alhydrogel (*73*). AMA1, a polymorphic MSP, is a leading asexual blood-stage malaria vaccine candidate. The overall structure of AMA1 appears to be conserved as compared to other surface proteins, but there are numerous amino acid substitutions identified among different *P. falciparum* isolates (*74-76*). This is the first reported use in humans of an investigational vaccine with the novel adjuvant CpG (cytosine and guanine separated by a phosphate) 7909. It is an immunomodulating synthetic oligodeoxynucleotide (ODN). CpG ODN as a vaccine adjuvant has a sequence of 5'-TCG TCG TTT TGT CGT TTT GTC GTT-3' with all nucleotides linked with phosphorothioate bonds and has been found to be considerably more potent than alum in mice. CpG 7909 is needed to induce high antibody levels for malaria proteins, which are generally poor immunogens in humans (*77*). As an adjuvant alhydrogel is known to

strongly promote Th2 (T helper)-type responses. Mullen and coworkers showed that the addition of CpG 7909 to AMA1-C1/Alhydrogel vaccine resulted in an increase in antibody titers and functional responses in mice, rats, and guinea pigs. The safety profile of the AMA1-C1/Alhydrogel® (aluminium hydroxide) plus CpG 7909 malaria vaccine is acceptable and a significant increase in immunogenicity was observed (*78,79*).

A Phase I trial of this vaccine in two different formulations (phosphate buffer and saline) and dosing intervals (1 month or 2 months) was conducted in healthy adults of age 18-50 years. AMA1-C1/Alhydrogel® + CPG 7909 in saline was shown to have similar immunogenicity as the AMA1-C1/Alhydrogel® + CPG 7909 buffered with phosphate and both the formulations were well tolerated. Excellent *in vitro* growth-inhibitory activity was reported in this human vaccine trial with AMA1. Based on the tolerability and immunogenicity of the vaccine a Phase II study suggested the development of a more immunogenic formulation of AMA1-C1
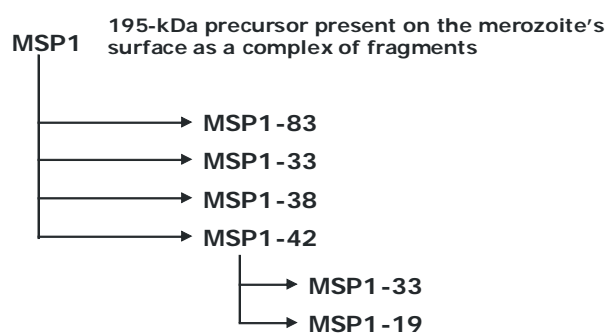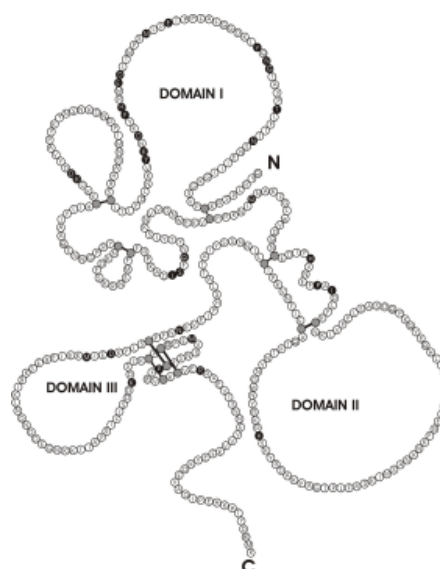

Figure 6. Proteolytic cleavage of MSPs.

Figure 7. Schematic of Pf AMA1 ectodomain (domains, I, II and III). Locations of eight disulphide bridges are shown in dark grey. D493, F505 and K544, are shaded in grey. Residues shaded in black represent mutations across 11 different naturally occurring *P. falciparum* sequences (*71,72*).

as an effective blood stage vaccine for *P. falciparum* malaria. Further, in the absence of a human blood stage challenge model or a predictive animal model, blood stage malaria vaccine development is empiric and demonstration of protection requires Phase IIb studies in the target population (*55*).

2.2.4. *Other blood stage antigens*

(*a*) *EBA-175 RII-NG* – One approach is to develop vaccines that target the molecular receptors used by the malaria parasite for the incursion of host cells and an intracellular translocation machinery to accentuate the process. The transmembrane erythrocyte binding protein-175 (EBA-175) and TRAP play a vital role in this course of action (*80,81*). The 175 kDa *P. falciparum* erythrocyte binding antigen (EBA-175) (Figure 8) binds sialic acid residues on glycophorin-A to mediate erythrocyte invasion. Two adhesive modules (called F1/F2) located in the extra cellular domain mediate the receptor interaction of EBA-175 (*82-85*). The binding region within EBA-175 is a cysteine-rich region identified as region II. Antibodies against region II block the binding of native EBA-175 to erythrocytes (*84,86*).

(*b*) *PfEMP1* – PfEMP1 (Figure 9) (*P. falciparum* erythrocyte membrane protein 1) is a high-molecular weight (200 to 350 kDa) transmembrane polypeptide consisting of 4 to 7 extracellular domains encoded by the *var* gene family. It has been identified as the rosetting ligand of the malaria parasite *P. falciparum* (*87,88*). PfEMP1 interacts with complement receptor, one on uninfected erythrocytes to form rosettes in the vasculature of the infected organ, and is associated with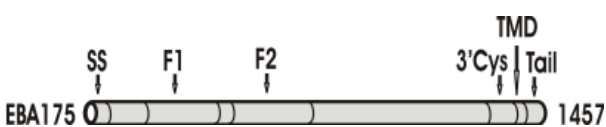 severe malaria. PfEMP1 could regulate both trophozoite and sexual stage densities (*89,90*). Immunity to the surface of the trophozoite-infected RBC could render protection from further infection. PfEMP1 appears to be the target of naturally acquired protective immunity in humans and immunization of *Aotus* monkeys with a domain of PfEMP1 inducing protection against a lethal *Plasmodium* parasite line (*91*).

(*c*) *PfEMP1 DBL1α-TM-AS* – The primary structure of PfEMP1 consists of a large N-terminal ecto-domain containing a variable number of DBL domains that mediate cytoadherence to various host cell receptors (*94,95*). The cytoadhesion of parasite-infected erythrocytes to a number of host cells is a causative factor in severe pathology of malaria, and PfEMP1 is considered the major virulence determinant of *P. falciparum* (*96*). Vogt *et al.* using recombinant proteins corresponding to the different domains of *P. falciparum* erythrocyte membrane protein 1 (PfEMP1) identified DBL-1α as the ligand for HS (heparan sulfate) (*97*).

2.2.5. *Combination blood stage vaccines*

(*a*) *PfCP2.9* – PfCP2.9 (Figure 10) is the first malaria vaccine candidate based on a chimeric recombinant protein vaccine for *P. falciparum* malaria consisting of AMA1 (domain III) and MSP1(19 kDa portion) expressed from the yeast *Pichia pastoris* with improved immunogenicity.

The enhancement of immunogenicity may be attributed to the presence of more T cell epitopes included in the construct and activity mediated by a combination of growth-inhibitory antibodies generated by the individual MSP1-19 and AMA-1(III) of PfCP-2.9 Vaccine (*98*). It was formulated with the adjuvant Montanide ISA 720 and at a dose of 50 μg, the vaccine appeared safe and highly immunogenic when observed during a preclinical evaluation (*99*).

A Phase I clinical trial was conducted at Shanghai, China to assess the safety, reactivity, and immunogenicity (antigen-specific antibody response) of PfCP2.9/ISA 720 vaccine. In this trial, volunteers developed high ELISA antibody responses to PfCP2.9

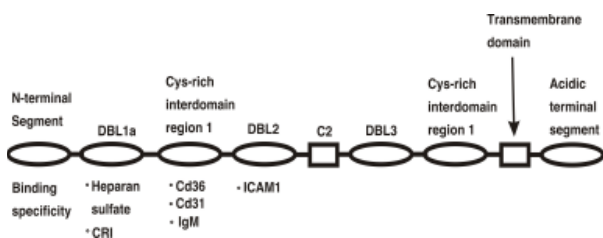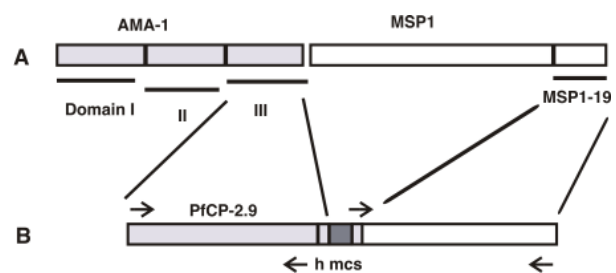**Figure 8. EBA-175 RII-NG-blood stage antigens.**

Figure 9. The multi-domain structure of *PfEMP1* (*91-93*). The extracellular domain building blocks: NTS, DBL, CIDR and C2 domains. Based on sequence similarity, DBL domains group as five types (α-ε) and CIDR domains as three types (α-γ)12. DBLα, β and δ types pair with other domains. Abbreviation: CR1, complement receptor 1; ATS, acidic terminal segment; CIDR, cysteine-rich interdomain region; DBL, Duffy-binding-like domain; ICAM-1, intracellular adhesion molecule-1; NTS, N-terminal segment; TM, transmembrane domain.

Figure 10. Schematic representation of the PfCP-2.9 gene construct and recombinant protein. (**A**) Localization of domain III of AMA-1 and MSP1-19 on the two molecules. (**B**) Structure of PfCP-2.9. The AMA-1(III) and MSP1-19 fragments were fused *via* a hinge-MCS (Multiple Cloning Site or polylinker)-hinge fragment (*98*).

but biological function of these antibodies was not reflected by *in vitro* inhibition of parasite growth. Additionally lower IFA (Indirect Immunofluorescent Assay) antibody responses were observed to native AMA1 and MSP1 in parasites (*100*). The most common adverse event was dose dependent reactivity at the injection site as reported earlier (*101*). Sinobiomed Inc., a Shanghai based leading developer of genetically engineered recombinant protein drugs and vaccines is planning to launch the Phase II clinical trial of its patented malaria vaccine candidate, PfCP2.9 (*http:// www.redorbit.com/news/health/1385194/phase_ii_clini cal_trial_of_sinobiomeds_malaria_candidate_vaccine_ to/*).

(*b*) *MSP3/GLURP (GMZ 2)/AlOH – Lactococcus lactis* expressed recombinant is a fusion protein, derived from *P. falciparum* Glutamate-rich protein (GLURP) genetically coupled to *P. falciparum* MSP3 and produced in *L. lactis* as a secreted recombinant GLURP-MSP3 fusion protein. It was designed with an aim to produce high levels of cytophilic antibodies. Antigenic studies suggested that the hybrid molecule provides an adequate presentation of GLURP and MSP3 antigenicity (*11,102*).

2.3. *Sexual-stage vaccines*

Induction of antibodies to gametocyte antigens can prevent fertilization in the mosquito; as well as its blood meal. The mosquito ingests antibodies that block fertilization. As a result, assessment of the efficacy of gametocyte vaccines is possible with a simple *ex vivo* assay. A sexual-stage vaccine consisting of an antigen not expressed in human beings during natural infection would not select for escape mutants. Therefore, combination of such a vaccine with a blood-stage or pre-erythrocytic vaccine could prevent potential immune selection. Sexual-stage vaccination would not protect vaccinated individuals from disease but would protect communities from infection.

2.3.1. *Pfs230*

The 230 kDa gametocyte gamete-specific surface protein of *P. falciparum*, Pfs230, is a target of antibodies which inhibit the development of the parasite inside the mosquito vector (Figure 11). A transmission blocking vaccine based on Pfs230 may be a powerful tool for malaria control. This sexual-stage falciparum surface antigen can elicit antibodies which block the infectivity of gametes in mosquitoes (*103-105*).

2.3.2. *Transmission-blocking vaccine*

Mosquito stage transmission blocking (MSTB) or transmission-blocking vaccine (TBV) (Figure 12) is an anti-mosquito stage vaccine that targets antigens

on gametes, zygotes or ookinetes. The idea for TBVs emerged from the 1976 observations of Gwadz (*106*) and Carter & Chen (*107*) which 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. TBVs do not confer protection to individuals and thus vaccination coverage will likely need to be widespread and sustained.

An underlying assumption of course is that proteins expressed in mammalian cells after immunization with DNA vaccine plasmids, are likely to be folded more like that in the eukaryotic parasite and thus elicit a functionally effective transmission-blocking immune response in experimental animals and ultimately in humans (*108,109*). The intention is to protect communities from infection, rather than the individual, but active clinical development of this approach is still awaited.

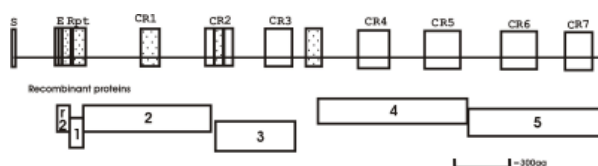The Phase I Trial of Malaria Transmission Blocking

Figure 11. Sequence domains of Pfs230 and the sequences from which recombinant antigens were derived. S, signal sequence; E, poly-glutamate region; Rpt, tetra peptide region; CR, cystein rich motifs; r2-5, recombinant proteins; Shaded area, net negative charge.
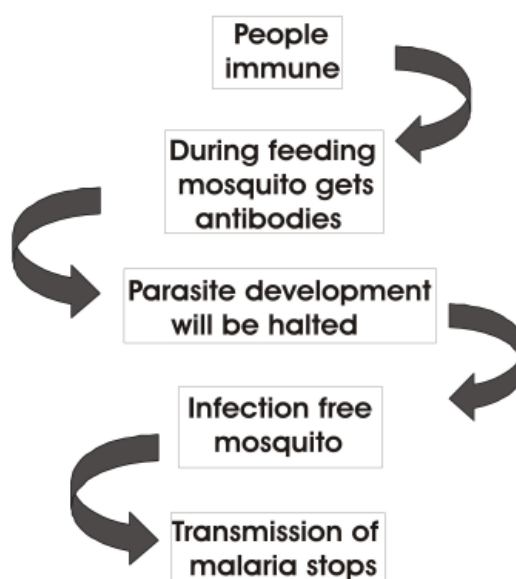

Figure 12. Concept of malaria transmission blocking vaccine Pfs25 and Pvs25.

Vaccine (TBV) Candidates Pfs25 and Pvs25 Formulated with Montanide ISA 51: Pfs25 and Pvs25, surface proteins of mosquito stage of the malaria parasites *P. falciparum* and *P. vivax*, respectively, are leading candidates for vaccines preventing malaria transmission by mosquitoes. It is feasible to induce transmission-blocking immunity in humans using the Pfs25/ISA 51 vaccine (*110*). Two TBVs, which were under clinical trial, withdrawn at Phase Ia were: Pfs25 ISA51 *Pichia pastoris* expressed, Pvs25 AlOH/ISA51 *Saccharomyces* expressed.

2.4. *Combination multi-stage vaccines*

Due to the complex life cycle and high antigenic diversity of the malaria parasite, a multistage vaccine may be necessary for optimal protection against the disease. A combination of pre-erythrocytic and blood-stage antigens is the most feasible approach to obtain a multistage vaccine to prevent malarial parasites from invasion of the host.

2.4.1. *FFM ME-TRAP + PEV3A*

A combination vaccine targeting different stages of the malaria life cycle may provide the most effective malaria vaccine. A new combination malaria vaccine is FFM ME-TRAP + PEV3A. PEV3A consists of peptide derived from both the pre-erythrocytic circumsporozoite protein and the blood stage antigen AMA-1 (*111*). Virosomal PEV3A vaccine given either alone or in combination with ME-TRAP vaccine had no protective effect in the malaria challenge model.

2.4.2. *NYVAC-Pf7*

A recently developed multistage vaccine, NYVAC-Pf7 is a single NYVAC genome containing genes encoding seven *P. falciparum* antigens. NYVAC-Pf7 is a genetically engineered, attenuated vaccinia virus, multistage, multicomponent *P. falciparum* vaccine that includes a TBV candidate Pfs-25, together with six additional leading candidate antigens (three pre-erythrocytic proteins: CS, SSP2/TRAP and LSA-1; and three asexual blood-stage antigens: MSP-1, AMA-1, and SERA) (*112*).

2.5. *T-cell targeting vaccines*

Most currently used vaccines work by getting the body to produce antibodies against the disease. Antibodies are unable to attack the malaria parasite once it has invaded liver cells and thus the approach was to design malaria vaccines that will produce potent T-cell responses against the liver stage of malaria infection (Figure 13). T-cells are a type of white blood cells called lymphocytes that circulate in the blood. There
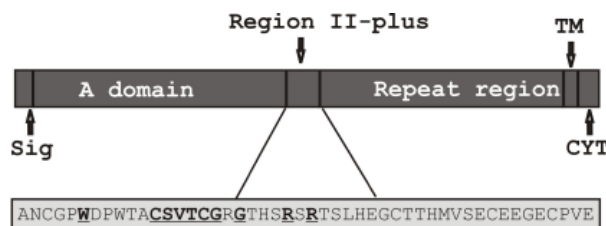

Figure 13. T-cell targeting vaccines.

are two types of T-cells: CD8+ T-cells and CD4+ T-cells and among them CD8+ T-cells play an important role in protective immunity. The vaccines stimulate populations of T-cells that will destroy liver cells that are harboring the malaria parasite and thus prevent parasite development. This approach could prevent both blood-stage infection and also prevent malaria transmission in endemic areas. The T-cells recognize the infected liver cells as they express small peptides from malaria on their surface (*113,114*). They also produce chemokines and cytokines particularly IFN-γ (Interferon-gamma) that can act in highly infection specific mechanisms to interfere with the spread and replication of a microbe. Mutation at a target epitope of the pathogen limits the ability of the T-cell (CD8+ T-cell) to recognize it and also in the presence of a high level of antigens, T-cells get exhausted after a certain time interval (*115*).

For many years the IFN-γ *ex vivo* ELISpot has been a major assay for assessing human T-cell responses generated by malaria vaccines. The ELISpot assay though a sensitive assay is an imperfect correlate of protection against malaria. Developing an ideal assay procedure was considered to be the major limitation for evaluating the efficiency of T-cell vaccines. Flow cytometric analysis of T-cell function that has been developed over time provides valuable insight into vaccine efficacy and protection (*116*). It also allows characterization of multifunctional antigen-specific T-cells following vaccination (*117*).

Flow cytometric analysis facilitates a more precise measurement of MIG (monokine induced by gamma) also known as CXCL9. Measurement of CXCL9, a chemokine induced by INF-γ, has now been considered as a more sensitive assay for the quantitative detection of INF-γ post vaccination (*118*).

2.5.1. *DNA vaccines*

The interest in this novel technology has been enormous and DNA vaccines are quite effective in priming immune responses and inducing immunological memory. Vaccine delivery systems that have been designed to induce protective CD8+ T-cell responses against *Plasmodium*-infected hepatocytes have been studied extensively but have been judged as being suboptimal for inducing protective immunity against

malaria. The key to success for any DNA-based therapy is to design a vector able to serve as a safe and efficient delivery system. Efficient and relatively safe DNA transfection using lipoplexes makes them an appealing alternative to be explored for gene delivery (*119*). The emerging technology of DNA vaccines offers many of the features desired in a malaria vaccine. Plasmid DNA introduced directly into the cells of the vaccine encodes antigen expression which stimulates a specific immunological response. This technique provides a practical and relatively simple approach toward designing a multivalent vaccine capable of delivering those antigens necessary to induce a protective immune response. This capability is especially relevant for a malaria vaccine. There are only a few target epitopes for T-cells and sequences vary from one strain of parasite to another and the specific variants do not often induce immunologically cross-reactive responses. Indeed, an effective DNA vaccine against malaria is expected to be a multi-gene vaccine (*56*). To date no DNA vaccine used alone has been reported to be efficacious in humans.

Although significant successes have been achieved in laboratory animal species, the level of immunity and protection afforded by DNA vaccines in larger animals and humans is often more limited than by conventional vaccines.

2.5.2. *DNA, MVA, and FP9 vaccines*

DNA, MVA and FP9 vaccines used initially encoded an identical DNA sequence consisting of a string of T- and B-cell epitopes from pre-erythrocytic antigens (the multi-epitope or ME string) fused to the entire sequence of TRAP (Figure 14) (*120-122*). TRAP is a pre-erythrocytic antigen that has been shown to be important for gliding motility and infectivity of liver cells and is currently being pursued as a malaria vaccine candidate for *P. falciparum* (*120,123,124*). It is a transmembrane protein that belongs to the TRAP/ Micronemal protein 2 (TRAP/MIC2) family required for sporozoite gliding motility and together with the circumsporozoite protein (CS) it has been found to be essential for the process of malaria sporozoite infection to the hepatocyte (*125-127*).

Several sequential clinical trials of DNA ME-TRAP, MVA ME-TRAP and FP9 ME-TRAP vaccines have been conducted to evaluate their safety, immunogenicity and protective efficacy in human volunteers. These vaccines are safe, highly immunogenic for CD4+ and CD8+ T lymphocytes and have shown encouraging and statistically significant results in studies of efficacy against a stringent, heterologous strain sporozoite challenge.

2.6. *Synthetic glycosylphosphatidylinositol (GPI) vaccine*

GPI (Figures 15 and 16), which is a pro-inflammatory endotoxin of parasitic origin and thought to be responsible for malarial acidosis, pulmonary edema and cerebral syndrome, has been synthesized. Hexasaccharide malarial toxin I synthesized by an automated procedure is currently under development as a malaria vaccine candidate (*129*). Using a combination of automated solid phase methods and solution-phase fragment coupling, the target GPI was assembled in a matter of days. Seeberger *et al.* recently reported the total synthesis of *P. falciparum* lipidated GPI by a highly convergent synthetic strategy. They placed three orthogonal protecting groups for the late stage installation of three lipid side chains on the GPI hexasaccharide backbone (*130*).

Synthetic GPI (*P. falciparum* GPI glycan) is a prototype carbohydrate anti-toxic vaccine, which could contribute greatly to prevent the pathology and fatalities of severe malaria (*131,132*).

Regardless of these advances, major improvements

$R^1 = C_{15}H_{31}$ $R^2, R^3 = C_{17}H_{35}$ **Various Lipid Chains**

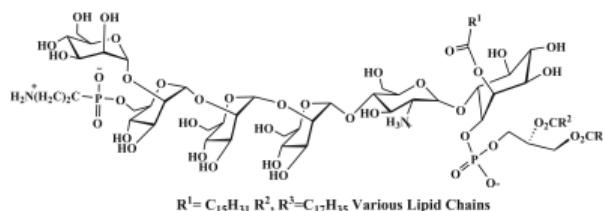**Figure 15. Consensus structure and retrosynthetic analysis of lipidates *P. falciparum* GPIs.**
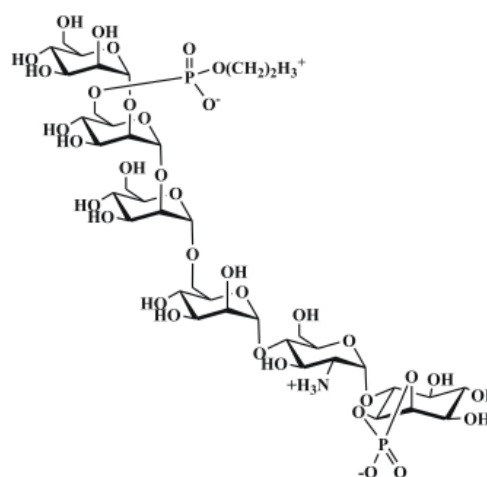
Figure 16. Hexasaccharide GPI malaria toxin-I.

Figure 14. Schematic representation of *Plasmodium vivax* TRAP protein. Bold and underlined characters represent the cell adhesive motifs. Sig, signal sequence; M, transmembrane sequence; CYT, cytoplasmic tail (*128*).

and innovative approaches are still needed. For many infectious diseases it is possible to produce an attenuated (harmless) version of the pathogen or a pathogen subunit that will lead to protective immunity without causing disease. Although in the case of malaria this is technically possible (irradiated malaria sporozoites given by infected mosquito bite can lead to protective immunity), it is impractical to do this on a large scale.

3. Conclusion

All vaccines which have failed in different phases or are in the pipeline toward success have been covered in this review. Many clinical trials and unresolved issues related to stability, immunogenicity and targeting to the site of the parasite life cycle give hope for further development of antimalarial vaccines. The development of a vaccine of therapeutic and protective benefit against the malaria parasite requires a novel approach and to date there are no vaccines available that can effectively target a parasitic infection. Traditional approaches to vaccine development against malaria have met with limited success. The search for an efficacious vaccine against malaria is ongoing and it is now widely believed that to confer protection a vaccine must induce very strong cellular and humoral immunity concurrently, but the vaccine, which has been promised to be 'just round the corner' for many years, remains elusive. Development of an effective and deployable malaria vaccine seems technically feasible in the view of most malaria researchers. New vaccine delivery methods and adjuvants could continue to increase the antibody and cellular immunogenicity of subunit vaccination. The development of a vaccine to protect human subjects against malaria is a feasible goal and the emergence of DNA vaccine technology offers a simple approach to formulating such a multivalent vaccine.

Highly purified subunit vaccines require potent adjuvants in order to elicit optimal immune responses and therefore an efficient adjuvant is also needed. A vaccine that would reduce both mortality and morbidity secondary to *P. falciparum* infection would be a valuable resource in the fight against this disease. A safe, effective and affordable malaria vaccine is expected to provide a long-lasting approach to prevent infection, reduce disease severity, prevent death and interrupt transmission. A vaccine that completely prevented infection, even for a relatively short time, would be very satisfactory for travelers.

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