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Malaria Vaccine Candidate Diversity Offers Challenges and Opportunities for Effective Vaccine Development

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Abstract

Malaria is one of the most deadly diseases caused by protozoan parasites of genus *Plasmodium*. It affects 300-500 million people annually, of which more than a million lives are lost; among them majority under 5 years of age. By conventional wisdom, the immune mechanisms responsible for protection against malaria will require a multiple of 10-15 antigen targets for proper protection against various stages of malarial infection. Such large number of targets cannot be delivered to humans, by this method. Moreover, each antigen is reported to be highly polymorphic in nature and the malaria-affected populations live in economically poor part of the world. Development of anti-malarial vaccines is therefore, a very tough challenge from technical, delivery and affordability points of view. Technical challenges include identification of epitopes / antigens against appropriate targets, construction of DNA vector(s) that will express properly folded functional protein. Vaccine delivery challenges include developing an easy method to deliver multiple doses within a short period of time to infants and children of less than five years of age. Affordability challenges include development of cost-effective vaccines that can be stored at room temperature and be easily delivered. Although the complex life cycle of Plasmodium is challenging for anti-malarial vaccine development, it also offers a lot of antigen targets (opportunities) to combat malaria. Information on anti-malarial vaccine candidates, DNA constructs and cost-effective delivery mechanism will be discussed.

Keywords: Malaria vaccine, *Plasmodium*, epitope, antigen, vaccine delivery

Introduction

Malaria is one of the deadliest diseases caused by *Plasmodium sp* and transmitted by female *Anopheles*. There are four different types of *Plasmodium* species causing malaria disease, namely *P. falciparum, P. vivax, P. malariae* and *P. ovale.* Among them, *P. falciparum* is considered the most dangerous and lethal for human beings. The two major malaria vectors in southern Africa are *Anopheles arabiensis* and *A. funestus.* On the other hand, *A. gambiae,* another malaria vector, can be found in the northern-most parts of this sub-region (Spillings *et al.,* 2008).

In 2006, there were approximately 247 million malaria cases among 3.3 billion people at risk, causing nearly a million deaths, mostly of children under 5 years old. One hundred and nine countries were endemic for malaria in 2008, 45 of which fall within the World Health Organization (WHO) African region (WHO report, 2008). According to WHO reports, in 2004, *P. falciparum* was considered one of the leading causes of death worldwide, induced by a single infectious agent. Both malarial parasites and insect vectors are increasingly becoming resistant to anti-malarial agents and insecticides (Chowdhury and Bagasra, 2007). Most malaria patients are treated with quinine, and combination of sulphadoxine and pyrimethamine (SP). However, *P. falciparum* is resistant to SP due to mutations of the dihydropteroate synthase (*pfdhps*) and dihydrofolate reductase (*pfdhfr*) genes in the parasite (Figueiredo *et al.,* 2008).

The *Plasmodium sp.* has a complex life cycle consisting of distinct phases (Boutin *et al.,* 2005), such as asexual (pre-erythrocytic and erythrocytic in humans), and sexual (in mosquito). The life cycle starts when a female mosquito, while feeding on the blood of a healthy individual, injects sporozoites of *Plasmodium* into the body (Girard *et al.,* 2007). In order to develop malaria, an interaction should occur between three populations: humans, *Anopheles* (vector) and the parasite. The parasite adapts through mitotic mutations and meiotic recombination to each one of these different hosts. It is assumed that diversity is the cause of a high variation in allele frequency at initial phase of mutant selection (Le Bras and Durand, 2003).

Until now, more than 80 malaria candidate vaccines developed against different stages *Plasmodium* life cycle have undergone preclinical trials of which approximately 30 undergone clinical trials and only two undergone phase 2b trials. Most extensive trial has been conducted on RTS,S, developed by GlaxoSmithKline (GSK) Biological where CSP antigen fused with Hepatitis B surface Antigen (HBsAg). This vaccine is now a candidate for phase 3 trial.

Although the complex life cycle of *Plasmodium* is challenging for anti-malarial vaccine development, it also offers many antigen targets (opportunities) to combat malaria. For example, the existence of the multistage life cycle parasite allows us to intervene at different stages of the parasite life cycle. The malaria spreading can also be stopped or significantly reduced by interrupting the normal life cycle of the parasite, including the period when the parasite is found in its vector (mosquito).

To effectively control the spread of malaria, it will be essential to create a multi-stage-specific complex vaccine or several individual stage-specific subunit vaccines singly or in combination, that could impede the malaria parasite development, if successful and would lead to vaccines that would interfere with the parasite when passing from one life stage to another. For an ideal malaria vaccine development, it is important to identify least polymorphic antigen(s) / epitope(s) that will induce maximum immunogenic response against majority of the parasites covering most diverse endemic areas. However, if such ideal antigen(s) / epitope(s) are not available, planning for a multi-component will be the next best approach. Therefore, it is important to compile malaria vaccine candidate antigen(s) / epitope(s) diversity information in one document that will be very useful for malaria researchers and also to speed up effective vaccine development against malaria. The focus of this review article will be to identify malaria vaccine candidate diversity within each stage of life cycle, allelic variation within each vaccine candidate, the type of challenges it generates and also the type of opportunity it offers to develop multistage-multi-antigenic vaccine to treat malaria effectively in an affordable manner. The vaccine candidate antigens / epitopes of each stages of life cycle are described below.

Pre-erythrocytic asexual stage vaccine candidate antigens

The malaria vaccine candidates against pre-erythrocyte stages include circumsporozoite protein (CSP) positioned intra-vascularly, the liver stage antigens (LSA-1 and LSA-3) which are intra-hepatocytic, and sporozoite surface protein-2 (SSP2) / Thrombospondin-related anonymous protein (TRAP) and CSP and TRAP-related protein (CTRP). Pre-erythrocytic vaccines play an important role in protecting against malaria infection and provide, in best cases, sterilizing humoral immunity by eliciting antibodies that target the invading sporozoites and preventing them from invading the liver (Girard *et al.,* 2007).

The CSP is a 58-kDa protein and denominates the major component of the surface of the sporozoite, the form of a malaria parasite (Chenet *et al.,* 2008). Before the initiation of the malaria infection, the mosquito injects the parasite into the vertebrate host. The CSP antigen is vital in protective immunity against the pre-erythrocytic stages of the *Plasmodium* infection (Kumkhaek *et al.,* 2005). The first *Plasmodium* gene to be cloned was a gene coding for this antigen, while the first *P. falciparum* subunit vaccine RTS,S (CSP fused with HBsAg) tested in human volunteers was also based on CSP. The vaccine RTS,S was tested on 215 malaria-naïve adults of Belgium and the US received 471 full doses (average efficacy 34% with a range of 8 – 53%) and on 266 adults in malaria-endemic areas of Gambia and Western Kenya received 782 full doses (average efficacy 71% with a range of 46-85%). The vaccine was also found safe as 53% efficacy was for 894 children of 5-17 months of age. This vaccine is now a candidate for phase 3 trial. Currently, CSP-based vaccines are considered as being the leading edge of malaria vaccine development and a potential vaccine candidate against *P. falciparum*. The CSP protein can be subdivided into two non-repetitive regions that exhibit polymorphisms and a variable central region consisting of several repeats of four-residue long motifs. Certain T-cell epitopes have been found in the non-repetitive regions, and immunodominant B-cell epitopes were observed in the central region (Chenet *et al.,* 2008). Apparently, *Pf*CSP's diversity is regionally restricted. Peru (Tab. 1), for example, has low genetic polymorphism with one predominant allele and variants in a small number, as in India, Vietnam and Thailand suggesting that since polymorphisms are restricted and can be grouped, allelic variants can be included in a polyvalent vaccine that could be widely effective (Chenet *et al.,* 2008).

Liver-stage antigen 1 (LSA1) is mentioned as the only *Plasmodium* antigen expressed in the liver-stages. The liver stage-specific antigen, LSA-1 is well conserved among *P. falciparum* isolates, being considered as one of the vaccine candidate (Chenet *et al.,* 2008). The LSA-1, a highly immunogenic gene in individuals naturally exposed to malaria, encoded a 200-230 kDa protein characterized by a large central repeat region spanning approximately 77% of the protein and flanked by short nonrepetitive N- and C-terminal regions. It has been shown that the protein is a target of B-cells, helper T-cells and major histocompatibility complex (MHC)-restricted $CD8^+$CTL (Ravichandran *et al.* 2000). T and B cell responses were also observed among populations living in malaria-endemic areas. Gambia was the first geographical area where naturally acquired reactions to LSA-1 peptides were involved in human protection. Further research of LSA-1 responses in different malaria affected regions also lead to the idea that LSA-1 responses are linked to the protective immunity of these naturally exposed people (Langhorne, 2005). LSA-1, as a single component vaccine, is supposed to elicit memory $CD8^+$ T cells present in the liver, and also secrete IFN-λ to confer protection, while IL-10 and the antibodies targeting LSA-1 should as well enhance natural protection and vaccine efficacy. Its reactions should be additive or synergistic to those elicited by other antigens seem that LSA-1 is more likely to be one element of a multi component vaccine (Kurtis *et al.,* 2001).

TRAP is an essential protein for sporozoite invasion (Weedall *et al.*, 2007). The TRAP protein can be found within the micronemes of the mosquito sporozoite stage. TRAP-deficient sporozoites are unable to invade mosquito salivary gland cells or liver cells, due to lack of gliding ability. It is considered that the cytoplasmic domain of TRAP is involved in connecting the parasite actin-myosin machinery with the external substrate (Gilberger *et al.,* 2003). However, CSP has also been identified as playing a crucial role in the invasion of hepatocytes by sporozoites (Gilberger *et al.,* 2003). Part of the TRAP sequence has homology to region II of CSP, between the A-domain and a repeat region. As opposed to an array of human T cell reactivity to epitopes in the C-terminal part of CSP, there appears to be largely distributed T cell reactivity to different parts of TRAP. Research conducted in Mali on TRAP did not reveal associations between the existence of TRAP polymorphisms and T cell epitope regions. Recent data however indicate that responses to allele-specific sequences may be common and could be further studied. Out of nine peptides representing polymorphic regions of TRAP studied, six (tp6, tp14, tp30, tp31, tp37 and tp38) contained sites identified by the F_{ST} analysis to be putatively under balancing selection (Weedall *et al.,* 2007).

CTRP is essential for the *Plasmodium falciparum*'s development in the mosquito's midgut. It also has an important role in the recognition and motility processes at the ookinete cell surface. Disruption of the *CTRP* gene by homologous recombination showed that *CTRP* has a crucial role in the development of *P. falciparum* for the mosquito midgut stage. When *Anophles* mosquitoes were fed with disrupted *CTRP* lines, oocyst formation was not observed despite the development of mature ookinetes. Developmental arrest of the human malaria parasite *P. falciparum* within the mosquito midgut was observed via *CTRP* gene disruption (Templeton *et al.,* 2000).

Erythrocytic asexual blood stream vaccine candidate antigens

Malaria vaccine candidates against erythrocyte stages includes an asexual blood stream stage developing in humans merozoite surface proteins (MSP-1, MSP-2, MSP-3, MSP-4); apical membrane antigen 1 (AMA-1); Erythrocyte binding antigen 175 (EBA-175); RAP1/2, Pf 35). Topolska et al. (2003) consider 10 different merozoite surface proteins, all present in Plasmodium Falciparum. However, these MSPs are not all present in all Plasmodium sp., as there are examples such as MSP-2 that is not present in Plasmodium Vivax, Plasmodium Knvoleski and Plasmodium Chabaud.

MSP-1, a 195-kDa protein, is another vaccine candidate for *P. falciparum*. Research on MSP-1 has proven that it can induce a protective immune response against infections caused by the parasite (Weiqing *et al.,* 1999). Before invasion, the MSP-1 protein is cleaved into an 83-kDa N-terminal fragment, two central fragments of 30- and 38-kDa and a 42-kDa C-terminal fragment. Just before invasion, the 42-kDa fragment is further cleaved into 33- and 19-kDa fragments (MSP-1_{33} and MSP-1_{19}). Block 2 is a principal target of antibodies within the 83-kDa fragment, while the MSP-1_{19} protein fragment becomes a major target of naturally-acquired anti-malarial immunity (Ferreira *et al.,* 2003) as it remains anchored to the merozoite surface at the time of erythrocyte invasion. Block 2 is associated with clinical immunity in African children (Chenet *et al.*, 2008). According to O'Donnell et al. (2001), block 2 is not the only putative target of protective antibodies within MSP-1 as there are other surface antigens involved in immunity against *P. falciparum* blood stages. It seems that the non-repetitive RO33-type version of block 2 is found in 45% of clinical isolates of *P. falciparum* examined in Brazil and only 11% in Vietnam, meaning that the presence of diversity in block 2 continues to affect the block after the acquisition of anti-malarial immunity. The immune evasion can also differ in populations, according to different areas of endemicity (Ferreira *et al.,* 2003). MSP-1 presents high antigen diversity. However, its polymorphism might become an obstacle for effective vaccines. The intragenic recombination between MSP-1 alleles is a major mechanism for the generation of allelic variation in MSP-1. This recombination occurs in the meiotic phase, in the vector mosquito gut, right after receiving blood containing male and female gametocytes, the frequency with which recombination occurs being highly important (Sakihamaa *et al.,* 2001).

Apical Membrane Antigen-1 (AMA-1) may also be considered as a sequence of the multisubunit vaccine for both *P. falciparum* and *Plasmodium vivax*, represented by an integral protein of 83 kDA with 16 cysteine conserved residues. As the AMA-1 vaccine has been proven safe and capable of inducing immunity in European adults, studies conducted in Mali in 2007 by African Malaria Network Trust confirmed its safety on African healthy population. Studies have shown that antibodies directed against AMA-1 neutralize the invasion of merozoites. Highly polymorphic with most of polymorphisms occurring in domain I, AMA-1 could hardly create a broadly effective vaccine. Its recombination, however, can induce protective immune responses in mouse (Mlambo *et al.,* 2006), and monkey models of malaria. Domain III of AMA-1, which is less polymorphic or non-polymorphic, constitutes the potential vaccine candidate, its monoclonal and polyclonal antibodies inhibiting the merozoite invasion of erythrocytes. Research conducted in the Wosera region of Papua New Guinea and focused on the AMA-1 diversity in a large number of symptomatic and asymptomatic *P. falciparum* infections brought new information of the malaria vaccine candidate's diversity in a single population. Various results indicated that polymorphisms in AMA-1 domain I are the result of selective pressure exerted by protective immune responses (Corte´s *et al.,* 2003).

Similar to the other vaccine candidates, EBA-175 also induces antibodies that inhibit malaria merozoite invasion. Mice cultivated antibodies against EBA-peptide 4 block binding of native EBA-175 to human erythrocytes and inhibit merozoite invasion *in vitro*. The antibodies against region II block invasion pathways that do not involve sialic acid. Biological and parasitological studies performed by Fousseyni *et al.* (2006) on 158 children from a village in southeast Gabon during the whole malaria transmission season of 1995 allowed the researchers to distinguish between protected and unprotected children. The study revealed that IgG antibodies competed for binding to EBA-175 epitopes and was consistent with the role of an isotype imbalance in the resistance and/or susceptibility to malaria infection. A different study conducted in three locations in Zimbabwe revealed that antibody levels against malaria antigens are distinct with regard to altitude and climatic conditions. Analysis revealed an age associated statistical significant pattern of reactivity to EBA-175, this finding is consistent with that of Okenu *et al.* (2000) and may suggest that antibodies to EBA- 175 may represent a marker of age acquired immunity. Antibodies that bind to EBA-175 may interfere with the interaction of EBA-175 with sialic acid residues of glycophorinA on erythrocytes, a crucial process in the sialic acid-dependent parasite invasion pathway (Mlambo *et al.,* 2006).

Transmission-blocking vaccines (TBV) candidate antigens

TBV are erythrocytic sexual blood stream vaccine candidate antigens. TBV can be divided into two target antigens: one pre-fertilization antigen and the other post-fertilization antigen. Pfs48/45 and Pfs 230 are pre-fertilization antigens found at the surface of the male and female gametes of malaria parasites. Pfs48/45 contains three of the cysteine domains responsible for male gamete fertility, while Pfs230 contains fourteen of the cysteine domains (Tsuboi *et al.,* 2003) of which every second domain is degenerate. The latter contains less than the full complement of six cysteines, but always an even number, such as four or two. Pfs48/45 and Pfs230 are ligands in the fertilization process (Carter, 2001). Monoclonal antibodies against these molecules can impede the transmission of parasites to mosquitoes. However, there is no evidence concerning the exact location of the target epitopes of the remaining monoclonal antibodies against either Pfs230 or Pfs48/45, except some monoclonal antibodies against Pfs48/45 that react with a known polymorphic epitope on this molecule (Carter, 2001).

Pfs 25 and Pfs 28 are post-fertilization antigens. The major advantages of post-fertilization target antigens are the strong immunogenicity and the limited pre-existing antigenic polymorphism (Tsuboi *et al.,* 2003). Surface proteins expressed on the zygotes and mature ookinetes of the malaria parasites represent the second class of malaria transmission (Carter, 2001). Two groups of molecules, the Pfs25 and the Pfs28, are currently considered as vaccine candidate antigens. The Pfs25 and Pfs28 proteins are rich in cysteines, but their arrangements are different compared to the first class proteins. They correspond to a series of four complete (six cysteines) or partial (four cysteines) EGF-like domains (Carter, 2001). According to Coban *et al*., antibodies directed against Pfs25 completely block pathogen transmission. Pfs25 material is being used for generating immune sera in mice and monkeys. When mixed with blood and cultured parasites and fed to mosquitoes through artificial membrane on the feeding apparatus, Pfs25 has potent transmission-blocking activity. At the same time, DNA encoding Pfs25, administered through the intramuscular route, is used to decrease the oocyst numbers in mosquito midgets by 97% through creating potent transmission-blocking antibodies in mice (Tsuboi *et al.,* 2003). Pfs28 is a 28-kDa protein, supposedly anchored to parasite surface by glycosylphosphatidylinositol, encoded on chromosome 10 and sharing putative functional determinants with Pfs25. Expressed on the

Tab. 1. Genetic Diversity of Vaccine Candidate Antigens. D1, D2 and D3 are domain 1, 2 and 3, respectively. The number in each box indicates the number of variable haplotypes of the parasite collected from patients in each area

Antigen type	Peru	Thailand	Nigeria	Papua New Guinea	Mali	Sri Lanka
CSP	3					
$MSP\text{-}1_{19}$					7	
MSP-1, Block-2	7	8				
MSP-1, Block-17	1					
MSP-2		11				
AMA-1	3	D-I =27	D-I = 45	D-I =27		D-I =17; D-II =13; D-III =2
LSA-1	3					
TRAP	1					

surface of late ookinetes, Pfs28 is involved in adherence to the mosquito's gut epithelium (Hisaeda *et al.,* 2000).

Multi-stage multi-epitope / antigen combination vaccines

Most of the candidate vaccines are based on different technological platforms such as recombinant proteins, synthetic peptides, viral vectors, bacterial vectors, plasmid DNA and attenuated organisms (Doolan and Stewart, 2007). Most of the vaccines under clinical trial are based on single antigen from a particular stage of *Plasmodium* life cycle. To effectively control the spread of malaria, it will be essential to creating a multi-stage-specific complex vaccine or several individual stage-specific subunit vaccines singly or in combination using most non-polymorphic region(s) of the promising candidate antigens. Several vaccines are developed recently based on the above idea and are listed below.

PfCP-2.9 is a vaccine candidate consisting of the C-terminal regions of two leading malaria vaccine candidates, domain III of apical membrane antigen-1 (AMA-1) and 19-kDa C-terminal fragment of the MSP-1. Both of these regions are least polymorphic portions of AMA-1 and MSP-1, respectively therefore most conservative antigens for combination vaccine candidate. It contains 18 cysteine residues, six in AMA-1(III) and twelve in MSP1–19, to form nine intramolecular disulfide bonds. The two candidates (AMA-1 and MSP-1) enhance the product yield and immunogenicity of the individual components when combined. The protective immune responses induced by either AMA-1 or MSP1–19 are dependent on protein conformation (Pan *et al.,* 2004). Pan *et al.* (2004) analyzed the chimeric PfCP-2.9 protein, a fusion of MSP1–19 and AMA- 1(III). In general, when two antigens are expressed together in a combination vector there may be antigenic competition. Fortunately, this vaccine candidate induces antibodies that inhibit blood-stage parasite growth *in vitro* and results showed no evidence of antigen competition. However, it revealed that sera from animals immunized with combination antigens recognized both the blood-stage parasite and the sporozoite. PfCP-2.9 chimeric protein previously appeared to be highly immunogenic in rabbits and monkeys. As the anti-AMA-1(III) antibody titer induced by PfCP-2.9 was 18-fold higher than that induced by AMA-1(III) and the anti-MSP1–19 antibody titer was 11-fold higher than that induced by MSP1–19 (Pan *et al.,* 2004), it was suggested that PfCP-2.9 in combination with these recombinant pre-erythrocytic antigens induced antibodies that inhibited growth of blood-stage malarial parasites. Responding to its potential, promise and prospect the Chinese government gave its largest ever biopharmaceutical grant to Sinobiomed for development of PfCP2.9 vaccine. The most promising home run drug in Sinobiomed's pipeline is its malaria candidate vaccine known as PfCP2.9, which targets the world's most deadly malaria parasite at its most destructive stage – the rapid replication in human red blood cells (*Investerms.com* Tuesday, September 16, 2008).

Although the RTS,S, a full copy of CSP-based subunit malaria vaccine is the most advanced at this time as per clinical trial concerns, the investigators in the field realized that the selected part of it has better potential as vaccine candidate. Therefore, recently PfCSP-C and PfCSP-RC are two recombinant antigens derived from the *Plasmodium falciparum* CSP were developed. *PfCSP-C* encodes a 112 amino acid portion of the C terminal region of PfCSP of the 3D7 line, while PfCSP-RC encodes a 201 amino acid portion of the central repeat region and flanking sequences of PfCSP of the same line.

Another leading candidate for the development of malaria transmission-blocking vaccines is represented by *Plasmodium falciparum* surface protein 25 (Pfs25) (Near *et al.,* 2002), a cysteine-rich 25-kDa surface protein with four tandem epidermal growth factor-like domains. Pfs25 begins with the gametogenesis in the mosquito midgut, continuing through zygote-ookinete transformation. Both P25 and P28 are related major ookinete surface proteins under consideration as candidates for inclusion in transmission - blocking vaccines (Tsuboi *et al.,* 2003). Prime vaccine candidates should include Pfs25 and Pfs28 as post-fertilization (zygote andookinete) stages of *P. falciparum*.

CDC/NIIMALVAC-1 is another considered candidate vaccine antigen, multistage and multicomponent recombinant malaria vaccine which proved to be highly immunogenic. It contains 12 B cell and 9 T cell epitopes derived from 9 stage-specific antigens. CDC/NIIMALVAC-1's advantage over vaccines based on single proteins is that multiple layers of protective immune responses cannot only prevent infection at multiple stages in the parasite's life cycle but can also help overcome problems associated with host genetic restriction of immune responses and genetic diversity in vaccine candidate antigens. The choice of the B cell-proliferative, T cell-proliferative and CTL epitopes used in the development of CDCyNIIMALVAC-1 was based on the results of immunoepidemiologic studies conducted in western Kenya (Shi *et al.,* 1999). Researches focused on the characteristics of naturally acquired immunity against infection and disease. After immunization of rabbit's with serum Igs, the vaccine elicited antibodies, which were apparently able to recognize various stages of the parasite, strongly inhibited parasite invasion, blocking the invasion of sporozoites into hepatoma cells. As expected, the vaccine-elicited antibodies produced significant anti-parasite activity against both sporozoite and blood stages of malaria, proving candidate's efficacy (Shi *et al.,* 1999).

To identify malaria antigens for vaccine development, Villard *et al.* (2007) selected 95 α-helical coiled coil domains of protein (motifs) predicted to be present in the *Plasmodium* parasite erythrocytic stage and tested their

corresponding chemically synthesized proteins. These proteins, in general, do not exhibit a folding problem, are a target of effective antibodies for several current malaria vaccine candidates. Indeed, the 95 chemically synthesized peptides were all specifically recognized by human immune sera, though at various prevalence. Moreover, these antibodies did not show significant cross reaction. The promising news is that it was demonstrated that the bioinformatics/chemical synthesis approach can lead to the rapid identification of molecules that target biologically active antibodies, thus identifying suiTab. vaccine candidates faster (Villard *et al.,* 2007).

The determination of the antigen's vaccine potential is highly dependent on its performance in biological assays that measure immunogenicity. Unfortunately, the relevance of these immunogenicity studies often remains unclear, comparisons between results from different laboratories often pursuing different formulations of the same antigen. Efforts are made to enable consistent inter-laboratory comparisons in order to enhance the predictability of assay data that would provide confidence in decision-making relative to choice of candidate vaccines. Examples of standardized comparative immunoassay are the growth inhibition assay (GIA), which measures the capacity of antibodies to limit the growth of *P. falciparum* in RBCs in culture and the Antibody-Dependent Cell-mediated Inhibition (ADCI) assay, which initially was developed at the Pasteur Institute in Paris (Girard *et al.,* 2007), assays that could be used in addition to ELISA.

Since majority of the people in the malaria-affected area are poor, any prevention measure or medication needs to be affordable by the affected population. Even if it is possible to develop most effective malaria vaccine, it will not be affordable by the affected population. Plant-based edible vaccine production is a paradigm-shift solution to the current bottleneck of vaccine supply as well as cost. With plant system, cost towards production and storage is significantly reduced. Moreover, risk of cross-contamination is also reduced significantly. In fact, this rapidly emerging technology is estimated to be a multi-billion dollar market soon (Joshi and Lopez, 2005). Due to its multi-host and multi-stage life cycle, the immune mechanisms responsible for protection against malaria will require a multiple of 10-15 antigen targets for proper protection against various stages of malarial infection (Chowdhury and Bagasra, 2007). By standard vaccination protocols, such a large number of targets cannot be delivered to humans. To circumvent these logistical difficulties and to deliver malaria vaccines to most of the susceptible patients at a fraction of a cost, anti-malaria edible vaccines in transgenic tomato plants (where different transgenic plants expressing different antigenic type(s), immunizing individuals against multiple antigens and against each stage of the life cycle of this multistage parasites) would be an efficient, inexpensive, and safe way of vaccination (Chowdhury and Bagasra, 2007; Chowdhury and Kantor, 2008).

Conclusions

Even after four decades of research effort to develop a malaria vaccine, until now although more than 80 malaria candidate vaccines have undergone preclinical trial of which approximately 30 undergone clinical trials, only two of them underwent phase 2b trial. This reflects the difficulty and complexity of malaria parasite life cycle and vaccine development challenges. Moreover, in the past, funding of this type of research was also a major stumbling block towards faster progress in this area. Fortunately, now with the Gate's Foundation's GCE funding and funding from China, there is a hope that progress will be fast and an affordable malaria vaccine will be available in the near future.

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