Review Article

Important advances in malaria vaccine research

Abstract

Malaria is one of the most widespread parasitic infection in Asian countries affecting the poor of the poor. In an effort to develop an effective vaccine for the treatment of malaria, various attempts are being made worldwide. If successful, such a vaccine can be effective for treatment of both *Plasmodium vivax* and *Plasmodium falciparum*. This would also be able to avoid complications such as drug resistance, resistance to insecticides, nonadherence to the treatment schedule, and eventually high cost of treatment in the resource-limited settings. In the current compilation, the details from the literature were collected by using PubMed and Medline as search engines and searched for terms such as malaria, vaccine, and malaria treatment. This review collates and provides glimpses of the information on the recent malaria vaccine development. The reader will be taken through the historical perspective followed by the approaches to the malaria vaccine development from pre-erythrocytic stage vaccines, asexual stage vaccines, transmission blocking vaccines, etc. Looking at the current scenario of the malaria and treatment strategies, it is an absolute need of an hour that an effective malaria vaccine should be developed. This would bring a revolutionary breakthrough in the treatment modalities especially when there is increasing emergence of resistance to existing drug therapy. It would be of great purpose to serve those living in malaria endemic region and also for travelers which are nonimmune and coming to malaria endemic region. As infection by *P. vivax* is more prevalent in India and other Asian subcontinent and is often prominent in areas where elimination is being attempted, special consideration is required of the role of vaccines in blocking transmission, regardless of the stages being targeted. Development of vaccines is feasible but with the support of private sector and government organization in terms of regulatory and most importantly financially, being an expensive venture.

Key words:
Infectious diseases, malaria, malaria vaccine, Plasmodium falcifarum, Plasmodium vivax

Introduction

Malaria is one of the most widespread infectious disease of human beings caused by infection with a parasite of genus plasmodium and transmitted by female *Anopheles mosquito*.[1] Malaria continues to be a major health problem in certain areas of world and is endemic particularly in the sub-Saharan Africa.[2] *Plasmodium vivax* infection occurs mostly in south East Asia. It is estimated that 300–500 million people become infected every year with very high rate of morbidity and mortality, with 1–3 million deaths occurring annually attributed to malaria, particularly among infants and children under 5 years of age.[3,4]

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Malaria infection causes significant economic losses and can decrease gross domestic product (GDP) by as much as 1.3% in countries with high levels of transmission.[5] Good control over incidence as well as transmission of the disease and also possible eradication is the need of hour. The treatment modalities for malaria include antimosquito approaches such as use of nets, sanitizers, and antiparasite approaches which include use of antimalarial drugs such as artimisinin derivatives, chloroquin, and primaquin. However, the treatment measures currently available do not suffice and are not adequate to control the disease; hence there is

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absolute need for developing an effective malaria vaccine.[6] The research community worldwide is engaged in the development of an effective malaria vaccine.[7] This malaria vaccine can avoid complications/problems such as high cost and resistance to antimalarial drugs, resistance to insecticides by vectors, uncertainties associated with larval control and poor compliance, and nonadherence to the control measures[8,9] associated with current treatment modalities. The recent statistics showed that there are more than 80 vaccines at preclinical development stage, above 30 in clinical testing while two have reached Phase 2b efficacy evaluation studies.[10] The key objective of malaria vaccine development is the induction of an effective pathogen-specific immune response that leads to protection against the infection.[11] In the current compilation, the details from the literature were collected by using PubMed and Medline as search engines and searched for terms such as malaria, vaccine, and malaria treatment. This review collates and provides glimpses of the information on the recent malaria vaccine development including the recent vaccine strategies and current preclinical and clinical vaccines under development.

Historical Development of Malaria Vaccine

The first malaria vaccine development was reported approximately a century ago.[6] However, due to complex nature of parasite and its life cycle, negligible progress has been made.[12] McGregor and Sydney Cohen in 1961 showed that children could acquire protection using gamma globulin component.[3,13] This vaccine was a single component vaccine. However, there was certainly a need to develop multicomponent vaccines which have both cellular and humoral components looking at the complex life cycle of the parasite. The Malaria Vaccine Technology Roadmap endorsed the concept of development of multistage, multiantigen, and whole organism malaria vaccines.[14] Their initiatives have resulted in greatly expanded investment in malaria vaccine research, which has resulted in the identification of a substantial number of vaccine candidates.[6] The countries with low and stable transmission rates have shown some promising research outcomes with respect to malaria vaccines.[15-19]

Approaches to Malaria Vaccine Development

Pre-erythrocytic stage vaccines

The primary objective of pre-erythrocytic stage vaccine is the provision of a level of protection that prevents any invasion of the blood and hence works on clinical malaria.[6] These are designed to target sporozoites or schizont infected liver cells and thus prevents the release of primary merozoites from infected hepatocytes.[6] Genetic attenuation of sporozoites is also being investigated.[20] Synthetic and genetically engineered subunit vaccines have been based mostly on two surface proteins, circumsporozoite protein (CSP) and as depicted in Figure 1 thrombospondin -elated

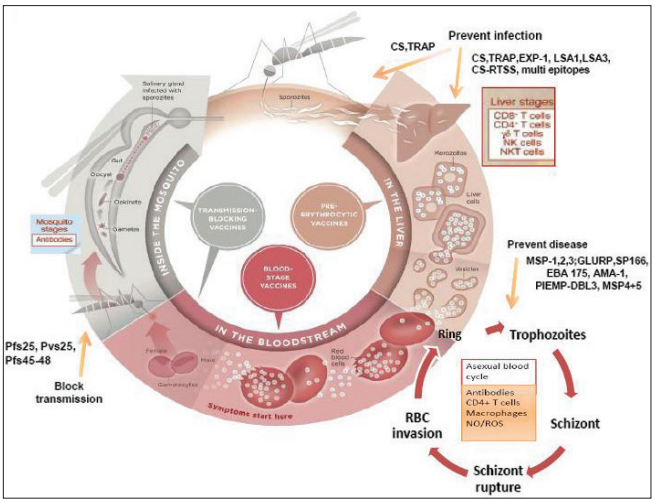

Figure 1: Illustration of malarial parasite lifecycle, approaches of vaccine development and examples of vaccines. Footnote: Modified from Editorial commentary by Breman JG, JID 2009:200, 317-320 (1 August) (Program for appropriate technology in health–malaria vaccine initiative)

anonymous protein (TRAP).[6] Vaccine candidate which has attracted many is RTS,S. This is a hybrid molecule and expressed in yeast, which consists of tandem repeat tetra peptide (R) and C-terminal T-cell epitope containing (T) regions of CSP fused to the hepatitis B surface antigen (S), together with unfused S antigen. The adjuvant ASO2 is a vital component of this vaccine.[6] The study of RTS,S/ASO2 in children in Mozambique showed some promising results; 30% protection against clinical malaria and 50% protection against severe malaria.[21]

Asexual stage vaccines

This is another strategy in the development of malaria vaccines. Principally, it has been observed that maternal antibodies passively transferred to the fetus may provide a window of protection against clinical malaria; again, a majority of individuals in endemic area acquire the ability to control parasite replication after repeated attacks of malaria. Also, hyperimmune globulin which can be prepared from the sera of individuals chronically infected with malaria can eliminate circulating parasites from *Plasmodium falciparum* infected individuals.[10] The principal target of current asexual stage vaccine development is merozoite, the stage that is initially released from the infected hepatocyte and rapidly invades and replicates in circulating red blood cells.[22]

Erythrocyte invasion is a rapid process and involves a number of parasite proteins that are located on the surface of the merozoites and are then available to circulating antibodies. Of these antigens, MSP-1, AMA-1, and MSP-3 have been produced as candidate malaria vaccines and have been shown to protect nonhuman primates from uncontrolled asexual stage parasitemia with the use of complete freund's adjuvant.[23] Another candidate for the malaria vaccines

has been a surface protein, parasite proteins present on the erythrocyte surface, such as PfEMP1. However, due to enormous antigenic variations, these would not constitute potential vaccine candidates.[24] Exception could be Var1CSA and Var2CSA members of PfEMP1 family that are being considered as candidates for use in a vaccine for pregnancy-associated malaria.[25]

Transmission blocking vaccines

Studies on animals have shown that it is possible to induce a highly effective transmission blocking activity.[26,27] Many of such vaccine trials include Pfs 48/45, Pfs 230, Pfs 25, and Pfs 28 of *P. falciparum*.[28,29] By using an *ex vivo* assay, it was possible to show transmission blocking activity of sera from vaccinated humans or animals. Experimentally, sera from rabbits, monkeys, and mice vaccinated with vaccine candidates Pfs25 from *P. falciparum* and pvs25 from *P. vivax* contained antibodies with transmission blocking activity. There was a correlation between the antibody levels and oocyst reduction and number of mosquitoes that failed to become infected;[30] also antibody levels remained at high levels for months after a second or third injection in mice.[31]

Antibodies obtained from animal vaccination and Phase 1 clinical trials could block the transmission of transgenic *Plasmodium bergehei* expressing the p25 antigens of either *P. falciparum*[32] or *P. vivax*.[33] This was an alternate approach to the membrane feeding assay for transmission blocking activity. There have been many parasite proteins identified;[28,29] however, most promising vaccine candidates for TBA which are of particular interest are *P. falciparum* Pfs 48/45 and Pfs 230 and their *P. vivax* orthologs which are expressed on both macrogametes and microgametes. An effective transmission reduction will be essential to meet the challenge of elimination of malaria disease leading to elimination and possible eradication from various endemic countries, as mentioned in the goals of the global malaria action plan of the Roll Back Malaria Partnership.[34] A vaccine targeting the process of malaria transmission could greatly facilitate achieving such goals.

Whole organism vaccine

Sporozoites

Over the years, studies using irradiated sporozoites have given us some valuable insights into the immune responses to the pre-erythrocytic stages of the parasite.[35] The attenuated-sporozoite vaccine is the most effective malaria vaccine tested to date.[36] A safe and protective attenuated-sporozoite vaccine was first administered to humans more than 30 years ago.[37] Reproducible data have subsequently been compiled to indicate that 92% of volunteers immunized with irradiated sporozoite were protected against parasite challenge.[38] Interestingly, unlike attenuated sporozoites, natural endemic exposure does not give rise to protective immunity,[39] possibly underlying the need for developmentally halted parasites. In fact, treatment to eradicate the irradiated forms from the liver ablates immunity.[40]

The effectiveness of targeting liver forms for induction of sterile immunity has also been pursued using normal sporozoite immunizations.[41] Such immunization, however, relies on the concurrent administration of chloroquine, an antimalarial that suppresses the blood stage but not the pre-erythrocytic forms. Interestingly, this approach was able to induce sterile protection and immune responses against liver and blood stages representing the first demonstration of cross-stage immunity induced by any vaccine approach. In another approach, 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.[38]

Large-scale production of sporozoites may lead to the development of an effective sporozoite vaccine. However, care has to be taken for the optimum radiation as 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 strategy to overcome this problem is to have genetically modified sporozoites, which can infect the hepatocytes, but get developmentally blocked postinfection.[20]

Erythrocytic stage

For blood stage vaccines, vaccination with low numbers of infected red cells is envisaged. Recently, it has been shown that repeated infection of naive 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*.[42] This immunity was predominantly mediated through proliferative T-cell responses, nitric oxide synthase activity, and interferon-g production in the absence of antibodies.[43] 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 needs to be worked very carefully.

DNA vaccines and live recombinant vaccines

Other vaccines based on the CSP antigen include plasmid DNA vaccines and live recombinant vaccines that use the attenuated modified vaccinia Ankara (MVA) strain, fowlpoxvirus, Adenovirus, Sindbis virus, yellow fever virus, or a cold-adapted attenuated influenza virus strain as a vector.[44] Some of these vaccines were tested together in prime-boost combinations.[45,46] Both the MVA and fowlpoxvirus recombinant vaccines were found to be safe and suitable for large-scale studies of children in Africa.[47,48]

The US Department of Defense, in collaboration with Vical, Inc., has developed candidate DNA vaccines for malaria (the Multi-Stage DNA Operation, MuStDO), including a liver-

stage DNA vaccine that encodes the CSP of *P. falciparum*. This DNA vaccine was tested in a "proof-of-concept" Phase I study carried out by the US Navy Malaria Program. The vaccine elicited cell-mediated immune responses but only modest antibody responses and no protection against experimental challenge in human volunteers.[49] Another vaccine called a multiple-antigen DNA vaccine, MuStDO-5, has been designed to encode five different liver-stage antigens: CSP, liver stage antigens 1 and 3 (LSA-1 and -3), exported protein 1 (EXP1), and the sporozoite surface protein 2 (SSP2, also known as thrombospondin-related adhesive protein, TRAP).

Various studies conducted in endemic areas have linked LSA-1 and LSA-3 with protective immunity. MuStDo-5 was manufactured as a combination of five separate plasmids. The vaccine, administered with GM-CSF DNA as an adjuvant, was safe and well tolerated in mice and rabbits[50] but showed only weak immunogenicity in primates and no evidence of protection was obtained in Phase IIa challenge trial.[51-53]

The Oxford University Malaria Vaccine Clinical Trials Group conducted studies of a DNA, a fowlpoxvirus, and a MVA-based vaccines expressing TRAP fused to a polyepitopic construct, demonstrating strong correlation between the induction of IFN-secreting CD4+ and CD8+ T-cell responses and protection against malaria in a mouse model.[54] The DNA and MVA candidate vaccines were combined in a prime-boost immunization trial in human volunteers in the Gambia.[55] No protection was observed against occurrence of disease, but malaria-related mortality was reduced.[53,55,56]

Thus, the most promising approach—heterologous prime-boost regimens, in which different viral vectors are sequentially paired with each other or with DNA or recombinant protein vaccines—is now being explored, and could provide high-grade protection, if findings in animal models are translatable to humans. Although a high safety concern remains as preexisting immunity to the vector particle and an unexplained safety signal observed in one trial suggesting an increased risk of HIV acquisition in volunteers with pre-existing immunity to the vector.[57]

Advancement of Malaria Vaccines in Different Phases

Preclinical studies on malaria vaccines

Pre-erythrotic stage (sporozoites and liver stage) vaccines are supported financially owing to their potential market in developed countries (armed forces, tourists, short-term visitors, such as business people and field researchers).[58] It was observed that vaccination of mice with irradiated sporozoites offered protection[59] and further protection could be achieved by immunization with the CS protein (CSP)[60] alone. This was a landmark observation from where the pre-erythrocytic vaccine took shape. Development of human pre-erythrocytic vaccines began with the cloning of *P. falciparum* CSP.[61] Currently, preclinical studies are aiming to induce higher levels of CSP-specific antibody. Conjugated CS repeat peptide to the mosquito stage ookinete surface protein Pfs25 induced long-lasting antibodies to both vaccine component in mice, conferring protection against liver infection and block transmission by mosquito vector.[62] Irradiated sporozoites were shown to be effective in mice[59] but had a major drawback that protection required the bites of more than 1000 infected, irradiated mosquitoes.[38] TRAP (thrombospondin-related adhesion protein), LSA (liver-stage antigen), and CSP were known targets of $CD8^+$ T cells which were induced in mouse model of malaria by immunization with irradiated sporozoites and killed parasite infected hepatocytes.[63] Yet immunization with viral vectors containing TRAP peptides produced low specific IFN-γ- producing T cells in naive adults.[64] Details of other vaccines in preclinical stage are detailed in [Table 1].

Clinical studies on malaria vaccines

In 1985, Walter Reed Army Institute of Research lead to development of RTS,S vaccine in a series of phase II clinical trials. 30–50% of malaria-naive adults immunized with RTS,S were protected against challenge by mosquitoes infected with the homologous *P. falciparum* clone.[65-70] In phase II field trials in the Gambia[71] and Kenya,[72] RTS,S conferred short-lived protection against malaria infection in approximately 35% of adults, although results from the Kenya trial did not reach statistical significance. Approximately 30%–50% of children and infants immunized with RTS,S in phase II trials conducted in Mozambique, Tanzania, and Kenya were protected from clinical malaria;[21,73-76] however, protection was generally short-lived. In field trials, immunization with RTS,S induced antibodies that correlate with protection from *P. falciparum* infection[77,78] but not clinical disease.[73,76,77] An ongoing phase III RTS,S/AS01 trial was conducted in seven African countries. 15,460 children from 6 weeks to 17 months of age were enrolled for vaccination with either RTS,S/AS01 or a nonmalaria comparator vaccine. The primary end point of the analysis was vaccine efficacy against clinical malaria during the 12 months after vaccination. It was found that the RTS,S/AS01 vaccine provided protection against both clinical and severe malaria in African children.[78] Another study assessed the efficacy of RTS,S/AS01E malaria vaccine during 15 months of follow-up in children from 5 to 17 months of age in Kenya and Tanzania. They concluded that RTS,S/AS01E confers sustained efficacy for at least 15 months and shows promise as a potential public health intervention against childhood malaria in malaria endemic countries.[79,80]

The first clinical trial with the irradiated, purified, cryopreserved sporozoite vaccine was safe and well tolerated, but only modestly immunogenic and protected only a few individuals. The next clinical trial will attempt to improve efficacy by optimizing the route of administration

Table 1: Overview of important advances in malaria vaccine research

Vaccine	Description	Current status	Remarks
Blood stage vaccine			
AMA-1-C1	Recombinant AMA1,FVO and 3D7, Alhydrogel	Phase I/II (Mali, 2–3 yr)	No efficacy[99]
FMP2.1	Recombinant AMA-1, 3D7, AS01B or AS02A	Phase I/IIa (US, adults), phase II (Mali, 1–6 yr)	No efficacy in *P. falciparum*–naive adults[119]
PfAMA-1-FVO	Recombinant AMA1, FVO, Ihydrogel, AS02A, or Montanide ISA 720	Phase Ib (The Netherlands and Mali, adults)	Safe and immunogenic in *P. falciparum*–naive adults[120]
AMA-1-C1	Recombinant AMA1, FVO and 3D7, Montanide ISA 720	Phase I (Australia, adults)	–
AMA-1-C1 + CPG	Recombinant AMA1, FVO and 3D7, Alhydrogel + CPG 7909	Phase I/IIa (UK, adults)	–
AdCh63 AMA1 and MVA AMA1	AMA1 expressed in AdCh63 (prime) and MVA (boost)	Phase I/IIa (UK, adults)	–
FMP1	Recombinant MSP1(21), 3D7, AS02A	Phase IIb (Kenya, 12–47 mo)	No efficacy[92]
MSP1(42)-C1	Recombinant MSP1(21), FVO + 3D7, Alhydrogel	Phase I (US, adults)	–
MSP1(42)-C1 + CPG	Recombinant MSP1(21), FVO + 3D7, Alhydrogel + CPG 7909	Phase I (US, adults)	Safe and immunogenic[98]
FMP010	Recombinant MSP1(21) FVO, AS01B	Phase Ia (US, adults)	–
AdCh63 MSP1 and MVA MSP1	MSP1 expressed in AdCh63 (prime) and MVA (boost)	Phase I/IIa (UK, adults)	–
BSAM-2	Recombinant AMA1 + MSP1(21), Alhydrogel + CPG 7909	Phase I (US and Mali, adults)	–
PfCP2.9	Recombinant chimeric AMA1 + MSP1[121], Montanide ISA 720	Phase I (China, adults)	Safe and immunogenic[100]
MSP3-LSP	MSP-3 long synthetic peptide, alum	Phase IIb (Mali,12–48 mo)	–
GLURP-LSP	GLURP long synthetic peptide, alum, Montanide ISA 720	Phase I (The Netherlands, adults)	Safe and immunogenic[91]
GMZ 2	GLURP + MSP3, alum	Phase I (Germany, adults; Gabon, adults and 1–5 yr)	Safe and immunogenic in *P. falciparum*–naive adults[90]
JAIVAC-1	MSP1[121] + EBA-175, Montanide ISA 720	Phase I (India, adults)	–
EBA-175 RII-NG	Recombinant EBA-175, aluminum phosphate	Phase I (US and Ghana, adults)	Safe and immunogenic in *P. falciparum*–naive adults[94]
SE36	Recombinant SERA5, alum	Phase Ia (Japan, adults) phase Ib (Uganda, adults)	Safe and immunogenic in *P. falciparum*–naive adults[97]
Combination B	Recombinant MSP1, MSP2, RESA, Montanide ISA 720	Phase I/IIb (Papua New Guinea, 5–9 yr)	↓ Parasite density;[93] no efficacy against blood-stage challenge in earlier trial[122]
Transmission-blocking vaccines			
PpPfs25	Recombinant Pfs25, Montanide ISA 51	Phase I (US, adults)	Immunogenic, but local and systemic reactogenicity[123]
Pfs25-Pfs25	Recombinant Pfs25 conjugated to itself	Phase I (US, adults)	–
Multistage vaccines			
PEV301 and PEV302	CSP and AMA1 mimetopes incorporated into influenza virosomes	Phase Ia (Switzerland, adults), phase Ib (Tanzania, 5–45 yr)	Safe and immunogenic[124,125]
PEV3A + FFM ME-TRAP	CSP and AMA1 peptides incorporated into influenza virosomes + FP9 ME-TRAP (prime) and MVA ME-TRAP (boost)	Phase I/IIa (UK, adults)	Possible ↓ blood-stage growth rate in some vaccines[126]
NMRC-M3V-D/Ad-PfCA	CSP and AMA1 encoded by DNA (prime) and expressed in adenovirus 5 (boost)	Phase I/IIa (US, adults)	–
NMRC-M3V-Ad-PfCA	CSP and AMA1 expressed in adenovirus 5	Phase I/IIa (US, adults)	–
FP9 PP and MVA PP	Six fused liver- and blood-stage antigens expressed in FP9 (prime) and MVA (boost)	Phase I/II (UK, adults)	No efficacy#

(Continued)

Table 1: Continued...

Vaccine	Description	Current status	Remarks
AMA1 MSP1 TRAP	AMA1 + MSP1, MSP1 + TRAP expressed in AdCh63 (prime) and MVA (boost)	Phase I/IIa (UK, adults)	–
P. vivax vaccines			
PvCS	*P. vivax* CS-derived long synthetic peptides, Montanide ISA 720 or 51	Phase Ib (Colombia, adults)	–
VMP001	*P. vivax* recombinant CSP, AS01B	Phase I/IIa (US, adults)	–
SPZ-Irrad	*P. vivax* live irradiated sporozoites	Phase I/IIa (Colombia,adults)	–
PpPvs25	Recombinant Pvs25, Montanide ISA51	Phase I (US, adults)	Immunogenic, but local and systemic reactogenicity[123]
Sporozoite and liver-stage malaria vaccines			
RTS,S	CSP coexpressed with HBsAg viral particles, AS01	Phase III (Burkina Faso, Gabon, Ghana, Kenya, Malawi, Mozambique, Tanzania; 6–12 wk and 5–17 mo)	Approximately 30%–50% efficacy in phase II challenge studies in US and field trials in Africa[119]
FP9 CS and CS MVA	CSP expressed in FP9 (prime) and MVA (boost)	Phase I/IIa (UK, adults), phase Ib (Gambia and Kenya, adults)	No efficacy[127]
PfCS102	Recombinant CSP, montanide ISA 720	Phase I/IIa (Switzerland, adults)	No efficacy[128]
ICC-1132	VLP consisting of HBc-expressing CSP epitopes, Seppic ISA 720	Phase I/IIa (UK, adults)	No efficacy[129]
Ad35 CS	CSP expressed in adenovirus 35	Phase I (US, adults), phase I (Burkina Faso, adults)	–
AdCh63 ME-TRAP and MVA ME-TRAP	ME-TRAP expressed in AdCh63 (prime) and MVA (boost)	Phase I/II (UK, adults)	–
FMP011	Recombinant LSA1,AS02A or AS01B	Phase I/IIa (US, adults)	No efficacy[130]
PfLSA-3-rec	Recombinant LSA3, alum, or Montanide ISA 720	Phase I/IIa (Netherlands, adults)	–
EP-1300	DNA polyepitope (CSP, TRAP, LSA-1, EXP-1) via electroporation	Phase I (US, adults)	–
PfSPZ	Radiation-attenuated sporozoite	Phase I/IIa (US, adults)	–
Pf GAP p52–/p36–	Genetically attenuated parasite; KO of sporozoite-expressed P52 and P36	Phase I/IIa (US, adults)	–
RTS,S/AS02D	CSP coexpressed with HBsAg viral particles, AS02D	Phase I/IIb trial in Mozambican infants (125)	–
RTS,S/AS01	CSP coexpressed with HBsAg viral particles, AS01	Phase III in seven African countries	Vaccine efficacy against severe malaria was 34.8% (95% CI, 16.2 to 49.2)[78]
RTS,S/AS01E	CSP coexpressed with HBsAg viral particles, AS01E	Phase III in Kenya and Tanzania (follow-up till 15 months)	Sustained efficacy for at least 15 months[79]

(S.L. Hoffman, personal communication). Studies are also in progress to determine whether sporozoites can be attenuated for use as vaccines by methods other than irradiation.[81,82,83] Immunization with viral vectors containing TRAP peptides led to partial protection in *P. falciparum*–naive adults from challenge by infected mosquitoes by mechanisms that involved the induction of large numbers of TRAP-specific IFN-γ-producing T cells.[63] However, this vaccine did not induce protection in children in Africa.[64] For unknown reasons, the level of TRAP-specific IFN-γ-producing T cells was considerably lower in vaccinated African children as compared with *P. falciparum*–naive adults.[63,64] Efforts to improve the T-cell immunogenicity of TRAP with simian adenovirus vectors are ongoing.[84]

For asexual blood stage vaccines, one of the important components is antibodies, as demonstrated by experiments in which the transfer of IgG from immune adult Africans to partially immune African[85] or Thai[86] children rapidly reduced parasitemia and fever. Volunteers repeatedly inoculated with *P. falciparum*–infected erythrocytes and then cured early in infection with antimalarial drugs were protected from reinfection.[42] Although antibodies to *P. falciparum* were not observed in the protected volunteers, there was a Th1-biased $CD4^+$ and $CD8^+$ T-cell response after exposure to malarial antigens *ex vivo*. However, the interpretation of this result is clouded by the possibility that the antimalarial drugs persisted at the time of challenge.[87] Thus far, relatively few blood stage antigens are in clinical development as vaccines.

These include apical membrane antigen 1 (AMA1),[88] erythrocyte-binding antigen–175 (EBA-175),[89] glutamate-rich protein (GLURP),[90,91] merozoite surface protein 1 (MSP1),[92] MSP2,[93] MSP3,[90,94-96] and serine-repeat antigen 5 (SERA5),[97] all of which are highly expressed on the surface of the merozoite. Unfortunately, recent phase II trials of the most advanced blood stage candidates, AMA1 and MSP1, did not demonstrate efficacy in African children.[87,92] Efforts to enhance the vaccine efficacy of AMA1 and MSP1 with novel adjuvants,[94,95] with viral vector prime-boost strategies,[84] or by combining AMA1 and MSP1[100] are ongoing. However, extensive parasite genetic diversity due to the selective pressure exerted by the human immune response presents a major hurdle for blood-stage vaccine development.[101-104] In a recent study, an array containing 1,204 *P. falciparum* proteins was probed with plasma from *P. falciparum*–exposed children in Mali to identify antibody profiles associated with naturally acquired malaria immunity.[105]

Obstacles in Malaria Vaccine Development

There have been many promising research observations in the malaria vaccine development; however, the disappointing results of clinical trials have resulted in reappraisal of current vaccine development strategies.[106] The biggest difficulties have been the parasite complex life cycle[107,108] at each stage, the parasite changes, its genetic profile,[109,110] and hence presenting varying antigenic agents to the immune system.[107] Stage-specific variation and the large size of the parasite's genome (~5500 genes),[107] many of unknown function,[111] have made identification of vaccine targets a real challenging task. Furthermore, challenges include that till date, despite having identified many vaccine candidates, none have reached for human use, provoking of best immune response has been defying, and also its difficulty in predicting the vaccine's success may be due to poor understanding of the interaction between parasite and human immune system.[112]

There have been many formulation challenges as well. Many adjuvants are being used for the delivery of antigens to the immune systems; however, there is considerable variability in the immune-enhancing effects of a given adjuvant.[113] Also, the incidence of severe or serious adverse reactions to an adjuvant is unpredictable.[114,115] There is a dire need for a single platform formulation, usable with a number of antigens, because the immunogenicity of each antigen in humans is influenced by the formulation used. The identification of potential formulations with low risks of adverse reactions will become a crux of the development when multiple antigens are spread in multistage combination vaccines. Also, there is need to expedite the human trials as the ongoing clinical trials at present are very few.

Conclusions

Efficacy of RTS,S vaccine (pre-erythrocytic vaccine) by delaying the first malarial symptoms[116] indicate the possibility that it reduces the number of infected hepatocytes, thus decreasing the number of merozoite releases into the blood stream, and allowing more time for blood stage immunity to develop before the fever threshold is reached. Thus, combining of *P. falciparum* antigens that target the pre-erythrocytic and blood stages may seem useful clinically.

The experimental malaria challenge model in humans using *P. falciparum*–infected mosquito bites is now well established in several international sites and increasingly used as a crucial check point for the clinical development of pre-erythrocytic stage malaria vaccines.[117] While requirement of high antibodies in blood stream[27] and effective for a vaccinated community and not directly to an individual, makes the development and implementation of transmission blocking vaccine difficult. Thus, the ideal strategy may be combination of pre-erythrocytic vaccine to prevent infection, such as the repeat region of the CSP, with a transmission-blocking vaccine, such as Pfs25.[62] Looking at the current scenario of the malaria and treatment strategies, it is the absolute need of an hour that effective malaria vaccine should be developed. Although development of malaria vaccines has its own limitations, its benefit would always have an upper hand over its drawbacks. Malaria vaccine development would bring a revolutionary breakthrough in the treatment modalities especially when there is increasing emergence of resistance to existing drug therapy. It would be of great purpose to develop malaria vaccines to serve those living in malaria endemic region and also for travelers who are nonimmune and coming to malaria endemic region. The subunit vaccine approaches have failed or conferred only limited protection; the delivery of an efficacious whole-cell, live-attenuated malaria vaccine is likely to be a critical tool.[118] As infection by *P. vivax* is more prevalent in India and other Asian subcontinent and is often prominent in areas where elimination is being attempted, special consideration is required of the role of vaccines in blocking transmission, regardless of the stages being targeted. Development of vaccines is feasible but with the support of private sector and government organization in terms of regulatory and most importantly financially as this venture is as huge demanding enormous amount of money for development.

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