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POTENTIAL ANTIGENTS FOR MALARIA VACCINE: A NEW HOPE FOR MALARIA ELIMINATION

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ABSTRACT

Malaria is a dangerous mosquito-borne disease caused by *Plasmodium* parasite. The disease threatens more than half of the world's population. Malaria prevention and treatment rely on synthetic medications, but the parasites have evolved resistance to all classes of drugs advised. This, together with the high prevalence of malaria in the developing world, highlights the urgent need for vaccine development. The purpose of this paper was to review the current level of knowledge on various *Plasmodium* antigens that can elicit the appropriate immune response. Several malaria vaccine candidates are moving forward, with more than 30 in advanced preclinical or clinical phases of development. However, only the RTS,S/AS01 vaccine has completed Phase III testing and got a favourable regulatory assessment. Pre-erythrocytic Vaccines (PEVs), Blood-Stage Vaccines (BSVs), and Transmission-Blocking Vaccines are the three types of malaria vaccines (TBVs). The majority of PEV research is currently focused on the development of subunit vaccines against parasite proteins such as the Pf circumsporozoite protein (PfCSP), the thrombospondin-related adhesion protein (TRAP), and the liver stage antigen (LSA). BSVs attack the parasite's blood stages. Two of the most likely BSV candidates are *P. falciparum* merozoite surface protein-1 (PfMSP1) and *P. falciparum* Apical Membrane Antigen-1 (PfAMA1). The most advanced contender is *Plasmodium falciparum* Reticulocyte-binding protein homolog 5 (PfRH5). TBVs interfere with the parasite's sexual development in the vector. Pfs25, P230, P48/45, Pfs2400, Pfg 27, Ps28, and Ps21 proteins are powerful antigens in TBVs because they are found at various phases of the parasite's growth while in the mosquito. With the availability of many various antigens, a malaria vaccine could be developed in the near future. More study into the practical application of these antigens is needed in order to produce a malaria vaccine quickly.

Keywords: Malaria, Vaccine, *Plasmodium falciparum*, Antigens, Antibodies

INTRODUCTION

Malaria is one of the most serious parasite infections worldwide, especially in tropical areas. It is caused by four species of the *Plasmodium* genus (*P. falcifarum*, *P.vivax, P.malariae* and *P.ovale*). *P. knowlesi* is a new *Plasmodium* species that has recently been discovered. Non-human primates are primarily infected by this species. *P. falciparum*, which is transmitted by female anopheles mosquitos during blood meals, causes the most lethal type of malaria. Malaria, HIV/AIDS, and tuberculosis are the three most deadly infectious diseases in the world (Yilong, 2011). According to the World Health Organization (WHO), the disease affects more than 3.5 billion people worldwide, with 300-500 million new cases

and 1.5-2.7 million deaths each year. Malaria kills one person every 30 seconds, making it a life-threatening parasitic disease, especially in the tropics, where 90 percent of the world's malaria cases are recorded (Patrick *et al.,* 2003). Malaria is without a doubt a disease of poverty, afflicting primarily the poor, who live in malaria-prone rural areas in poorly-constructed shelters with few, if any, barriers against malaria vectors (Adewole, 2012).

P. falciparum induced malaria generates severe malaria that can cause unconsciousness and even death in a short amount of time, making it one of the most dangerous forms of malaria. It is also the most common and leading cause of mortality worldwide (World Health Organisation, 2015). The main cause of this serious complication in *P. falciparum*-induced malaria is cytoadherence, which is the attachment of infected red blood cells to the vascular endothelium, resulting in a reduction in the size and destabilisation of small blood vessels, as well as a reduction in blood flow to and malfunctioning of some sensitive organs such as the brain and spinal cord (Olatunji, 2018). Fever and chills are two of the most common symptoms of malaria, both of which are caused by a reaction to malaria antigens. Other symptoms include anemia, which is caused by intravascular rupture of the red blood cell, and extra-vascular haemolysis, which is caused by the sequestration of parasitized and non-parasitic red blood cells.

With the emergence of drug-resistant *Plasmodia* strains, the efficacy and therapeutic benefits of chemoprophylaxis and chemotherapy, which rely only on synthetic antimalarial medicines, are dwindling. This, together with the high number of new cases of malaria reported in developing countries, particularly Nigeria, highlights the urgent need for a paradigm change away from chemoprophylaxis and chemotherapy and toward vaccinations (Veronique *et.al*.,2014). As a result, an efficient antimalarial vaccine is required that evokes long-lasting immune responses capable of managing and preventing the disease's incidence and spread (Peifang et.al.,2003). This is due to the fact that they are considered one of the most promising techniques of managing infectious diseases such as malaria, particularly in Sub-Saharan Africa (Maureen et.al,2004). In some circumstances, vaccines are seen to be the most cost-effective way to combat infectious disease (Zhangping et.al.,2018).

One of the most important benefits of an efficient malaria vaccine is that it protects the vulnerable population (Michael et al., 2015), particularly children under the age of five and pregnant women with a weakened immune system. The premise behind malaria vaccines is that, like all other vaccinations, they cause the production of antibodies that impair or destabilize some essential parasite growth process. For instance, sporozoite invasion of the liver and merozoite invasion of the red blood cell, as well as other parasite survival mechanisms such as rossetting and cytoadherance, as well as interfering with the parasite's sexual development in the mosquito's midgut. As a result, the goal of this paper was to gain insight into several potential *Plasmodium* antigens at various developmental stages that could evoke a rapid and vast immune response when the parasite was assaulted.

MALARIA VACCINE DEVELOPMENT

In comparison to bacterial and viral vaccinations, the development of a malaria vaccine has been lengthy and difficult. This significant difference is due to the malaria parasite's genome, which is significantly larger and more complex (14 chromosomes with nuclear, Plasmid, and mitochodrial genomes) than bacterial (single chromosome) or viral genomes (Kaushik and Sunanda,

2019). For this and other technical reasons, malaria vaccine development began more than 50 years ago and there is still no licensed product. Despite the numerous bottlenecks associated with malaria vaccine development, most experts believe that malaria vaccines will be biologically achievable in the near future. This is due to the fact that persons who have been exposed to the disease (Malaria) for an extended length of time have a natural immunity against it. Such immune individuals, in most situations, house few parasites, resulting in a very low degree of parasitaemia, and thus a very low risk of becoming ill as a result of the disease (Trevor and Stephen,1994). However, despite all of these expectations and analyses, there is no universal agreement that immune responses to exo-erythrocytic immune gained through natural exposure, whether antibody or cell-mediated immunity (CMI), contribute significantly to naturally acquired immunity, but the attenuated sporozoite model shows that immune responses to sporozoite and liver-stage parasites can protect humans (Lauren et.al.,2012).

Years later, attenuated sporozoite implanted in mice became the basis for the present malaria vaccine. As a result, there is a larger chance that a licensed, partially effective vaccination with a short duration of action will be available shortly (USAID,2016). According to Carlota and Joseph, in 2009, the Malaria Vaccine Initiative (MVI) and the Bill and Melinda Gates Foundation established a new and achievable plan targeted at developing a licensed new malaria vaccine with relatively high efficiency (at least 80%) by the year 2025.

A large number of *Plasmodium* antigens from various stages of the parasite's development have been discovered, and some of them are now being tested for the creation of a malaria vaccine. Through that end, there are around thirty malaria vaccine candidates in various phases of development, ranging from advanced preclinical to clinical trials. Among these, only the RTS,S/AS01 vaccine, produced by GlaxoSmithKline (GSK) in 2015(Julia et al.,2018) has passed Phase III evaluation and has demonstrated to be a very good candidate for malaria vaccination, receiving a favourable regulatory assessment as a result (WHO,2016; Simon et al., 2018).

Protective immunity is thought to be mediated primarily by humoral and cellular immunity, with high antibody titers, which can totally stop liver infection caused by sporozoite invasion (Desalegn, et.al.,2016). As a result, the vaccine (RTS,S/AS01) which is a single-bond recombinant protein (Neafsey et.al.,2015) generates humoral and cellular immune responses to the Circumsporozoite Protein, which is found on the surface of sporozoites and liver stage schizonts (Shima and Hossein, 2017). As a result, the vaccine (RTS,S/AS01) prevents *Plasmodium falciparum* from entering the liver, preventing sporozoite development into deadly erythrocyte stages.

The principal active biomolecular component of this vaccine is the parasite's circumsporozoite protein (CSP), which is obtained from the sporozoites' outer surface (Veronique, et.al., 2014). It contains the carboxyl terminus (amino acids 207– 395) of the *Plasmodium falciparum* circumsporozoite (CS) antigen, which was genetically modified. As a result, the RTS,S/AS01vaccine focuses immune responses on the primary circumsporozoite protein present on the infecting sporozoite's surface (Simon et.al.,2018). This protein is involved in the attachment of the sporozoite to the hepatocyte and subsequent invasion of the hepatocyte during the sporozoite and early liver stages of parasite infection. Anti-Circumsporzoite antibodies have been proven to interfere with

parasite invasion processes and to be linked to a lower chance of clinical presentation of illness symptoms and/or the disease itself (Lauren et.al.,2012). Hepatitis B surface antigen virus-like particle (VLP) is another active biomolecular component of the vaccine; the hybrid malaria-hepatitis B VLP is manufactured with a mixture of liposomes (MPL and QS21) as adjuvant (GlaxoSmithKline's AS01 adjuvant).

The development of RTS,S provides a unique opportunity to gain a comprehensive understanding of the technical, clinical, guideline, and conditional components of malaria vaccine development from regulatory agencies (Christian,2010). Between 2009 and 2014, Glaxo Smith Kline (GSK) and the Malaria Vaccine Initiative (MVI) conducted a large-scale Phase III clinical trial in seven African countries, including a large network of research centers at 11 sites (Kaushik and Sunanda,2019) (Geoffrey,2005).

However, one key truth about any malaria vaccine is that it is unlikely to be perfect enough, and research into the decision to launch a flawless and actual vaccine will create new issues that may necessitate more advanced analysis. For example, such new research and development could make the vaccine very expensive per dose (Melinda and Sarah, 2004). Another obstacle to malaria vaccine development is the lack of a traditional market for the vaccine. This is due to the fact that malaria disproportionately affects the poorest countries, which lack the financial resources to purchase vaccines, and vaccine manufacturers have little incentive to develop vaccines for this disease. However, during the clinical trial, some of the RTS,S vaccine's major flaws were noted, including an increase in the incidence of meningitis cases in vaccinated children, which was thought to be an adverse effect of the vaccine, and "age shift," which is the waning of vaccine efficacy over time, resulting in a rebound of malaria cases (Kaushik and Sunanda,2019).

TYPES OF MALARIA VACCINE AND POTENTIAL ANTIGENS

The types of malaria vaccines available are determined by the sort of prevention offered or the developmental stage of the parasite targeted by the vaccine, and this also influences the vaccine development tactics. In most cases, malaria vaccines aim to target three key stages in the parasite's development: first, vaccinations that target and kill sporozoites when they enter the bloodstream, preventing them from infecting the liver. This vaccination has the potential to totally prevent illness. Second, vaccines that would protect against the parasite's blood stages. These vaccinations can only prevent infection by limiting the invasion of erythrocytes and causing moderate illness. Finally, transmission blocking vaccinations induce the formation of antibodies against the parasite's sexual stages, leaving the parasite sterile within the mosquito and preventing it from infecting a new host during a blood meal (Patrick,2007). As a result, malaria vaccinations are divided into three categories: exoerythrocytic vaccines, blood-stage vaccines, and transmission-blocking vaccines, and long-term protection necessitates a combination of vaccines that may target various stages of the parasite's development (Peng,2017).

Exoerythrocytic Vaccines (Pre-Erythrocytic Vaccines (PEVS))

Because pre-erythrocytic malaria is clinically silent and lasts only a few days in most *Plasmodia* species, it is a particularly appealing target for protective immunity (Shobhona and Sulabha,2008; Kirsten et.al.,2018). This is because they can prevent initial infection and thus clinical illness and

malaria transmission (James et.al., 2019), and thus it is regarded as an ideal candidate for malaria vaccine. This is often accomplished using a variety of whole-sporozoite and/or subunit vaccination methods. The latter entails inoculation with sporozoites during chemoprophylaxis and vaccination with live sporozoites attenuated by radiation or genetic alteration (Katharine et.al.,2017). RTS,S is an example of subunit pre-erythrocytic vaccine, in African newborns, however, it provides only modest and transient protection (Susanne et.al.,2014).

The pre-erythrocytic stage vaccine (PEV) targets sporozoites and liver stage parasites, blocking parasitic progression to blood stages and thereby preventing sporozoites from entering liver cells and maturing into tissue schizonts. The vaccines can also target antigens expressed during the early and late stages of liver schizogony, which may induce cell-mediated immunity and inhibit intracellular parasite growth. This vaccine may also be perfect for targeting *P.vivax's* latent phases (hypnozoites), which are responsible for the infection's recurrent nature (Desalegn et al., 2016). To have a true protective effect, vaccines targeting pre-erythrocytic *Plasmodium* must have a 100% protection rate. *Pf circumsporozoite protein (PfCSP)*, *Thrombospondin-Related Adhesion Protein (TRAP),* and *Liver Stage Antigen (LSA)* are the major parasite proteins that are currently being considered as potential candidate antigens for the development of a pre-erythrocytic vaccine (Jingtong et al.,2019).

Blood Stage Vaccines (Erythrocytic Vaccine)

Blood-stage vaccines are vaccines that are designed to target and destroy the parasite's erythrocytic stages, reducing morbidity and mortality. They also have the added benefit of being multistage and multi-antigen vaccines (Muhammadou, et.al.,2011). The main goal of the Erythrocytic vaccine is to prevent the parasite from developing in the bloodstream by interfering with the normal erythrocyte invasion process. This is because merozoite invasion of erythrocytes is one of the most important factors in malaria pathogenesis. Apical Membrane Antigen-1(AMA1), a critical invasion protein of merozoites and the most investigated potential protein for this stage vaccination, is the most common candidate protein (James et.al., 2019). Apart from AMA1, a small number of candidate proteins have progressed to phase I and II clinical trials for evaluation. While such vaccines are unlikely to prevent infection, they may be able to reduce parasite burden, the duration of clinical disease, and/or the severe consequences of malaria, such as mortality.

P. falciparum merozoite surface protein-1 (PfMSP1) (James et al.,2013) is another prospective protein for a blood-stage vaccine. PfMSP1 is a key protein component present on the surface of the merozoite stage of all *Plasmodium* species. This protein (MSP1) is important in the invasion process because it goes through two distinct proteolytic maturation steps during merozoite development that are required for erythrocyte invasion. Antibodies that target MSP1-19, a C-terminal maturation product, can stop erythrocyte invasion and parasite growth. As a result, this polypeptide is regarded as one of the most promising malaria vaccine candidates (Pizarro et.al.,2003). However, due to high levels of polymorphism and redundancy in previous candidate antigens, non-protective or strain-specific vaccine-induced antibody responses have resulted (Jing et.al.,2018).

Plasmodium falciparum Reticulocyte-binding protein homolog 5 (PfRH5) has recently been identified as a novel promising candidate protein for a blood-stage vaccine, owing to its

high conservation and resistance to vaccine-induced antibodies. The disease-causing blood-stage of malaria is the primary target for next-generation vaccinations (Daniel et.al.,2019; Jing et.al.,2018). Table 1 shows the potential antigens for a blood-stage vaccination in detail.

Transmission-Blocking Vaccines

Malaria control techniques have always included measures to prevent transmission. The Malaria Vaccine Technology Roadmap's amended 2030 Strategic Goal now calls for the development of vaccinations that minimize transmission, decreasing incidence and enabling elimination in multiple settings (Yuanyuan et.al.,2015). Malaria Transmission Blocking Vaccines (TBVs) could be another efficient way to prevent malaria transmission by targeting the parasite's sexual development in the mosquito vector. Overall, when employed as part of a control program, a sustained reduction in transmission could help to ensure the disease's local eradication (Yimin et.al.,2008). In addition, combining transmission blocking vaccines (TBVs) that inhibit disease spread with drug-based therapy to treat sick individuals is a potential malaria treatment technique.

TBVs are developed against parasite surface protein antigens that are normally expressed during parasite sexual reproduction in the mosquito, so that when the mosquito ingests blood from a vaccinated person carrying the *Plasmodium* parasite, the antibodies prevent the parasite from completing its life cycle (Carla et.al.,2012). As a result, combining Pre-erythrocytic Vaccines (PEVs) with Transmission Blocking Vaccines (TBVs) could improve their efficacy, as TBVs lower the amount of parasites infecting each mosquito. This means that fewer parasites are injected into the next person, limiting the quantity of parasites that Pre-erythrocytic Vaccines can manage. PEVs work better when there are fewer parasites infecting a person at any particular time, according to research (Ellie et.al., 2018).

Tables 1 and 2 indicate the numerous possible proteins, as well as their location on the parasite and method of action. Pfs25 and Pvs25 are two candidate proteins for Transmission Blockin vaccines, with Pfs25 being the most promising. In the mosquito stages of *P.falciparum* and *P. vivax,* these proteins are expressed on the surface of zygote and ookinetes, respectively (Yuanyuan et.al.,2015), P230 and P48/45 are two other potential candidate proteins. These proteins are the best-characterized gametocyte membrane proteins that are exposed after gamete emergence from the Red Blood Cell. Additional potential proteins include P230 and P48/45, which are both expressed at the gametocyte and gamete stages of development. Furthermore, the mosquito midgut induces the expression of extra gamete surface proteins from mRNA stored in the parasite's cytoplasm (Acquah et.al.,2019).

Other gametocyte, gamete, zygote and ookinete surface proteins Pfs2400, Pfg27, Ps28, have been identified as potential antigens. Other epitopes from later stages (Ps21) contain molecules such as chitinase, which is important for Peritropic Membrane and midgut traversal, and Alanyl Aminopeptidase (AnAPN1), an antigen in the midgut surface, important for ookinete recognition, were identified as potential targets (Daniel and Parick,2016). The diagram below depicts the development stages of *P. falcifarum* in mosquitos, as well as potential antigen targets for a Transmission Blocking Vaccine. The table 1 and 2 below depicts the various vaccine classes and their potential vaccines.

CONCLUSSION

Malaria vaccine is very likely in the near future due to the availability of different potential antigens at different developmental stages of the parasite, ranging from sporozoite in the blood and liver, merozoite in erythrocytes, and zygote and ookinete in the vector. In addition if required result from the use of malaria vaccine is to be achieved, all three classes (Pre-erythrocytic, Erythrocytic and Transmission Blocking Vaccine) of vaccines need to be available in order to drastically reduce or completely render the parasite incapacitated. Successful implementation of malaria vaccine may completely prevent the problem of resistant by the parasite as a result of massive administration of synthetic antimalarial drugs.

Recommendation

Further research into the practical application of these antigens is needed in order to fully realize the potential of these proteins in the creation of malaria vaccines. Furthermore, more study should be carried out in order to find new potential proteins, particularly those that are relevant in the development of transmission-blocking vaccines.

Table 2: Key antigens in *P. falciparum* gametocyte development and transmission-blocking vaccines

Antigen(s)	Localization	Function
PfEMP-1	RBC membrane	Sequestration
RIFIN	Unknown	Sequestration?
STEVOR	RBC membrane	Sequestration?
Pfs16	ParasiphorousVacoule Membrane	Unknown
Pfpeg3 and -4	ParasiphorousVacoule Membrane	Early gametocyte development
PF14 744 and PF14 748	ParasiphorousVacoule Membrane	Gametocyte development
Pfg377	Osmiophilic bodies	Gametocyte maturation, osmiophilic body formation, and egression of macrogametes
Pfg27	Gametocyte cytoplasm	Gametocyte formation/integrity; evidence for RNA binding
Pfs48/45*b*	Gametocyte/Gamete Membrane;	Fertilization: male fertility factor attachment of male microgametes to fertile female macrogametes
Pfs47	Gametocyte/Gamete Membrane	Fertilization: female fertility factor (not essential)
Pfs230*b*	Gametocyte/Gamete Membrane	Fertilization: adherence of male gametes to red blood cells; may protect parasite from contents of blood meal; complement required for antibody function
HAP-2	Gametocyte/Gamete Membrane (male)	Fertilization: fusion of gamete surface membranes
Pfs25 and Pfs28*b*	zygote and ookinete membrane	Midgut penetration, ookinete survival, oocyst formation

Table1: List of *Plasmodium* vaccines in pre-erythrocytic stages, blood stages, and transmission-blocking stages

S/N	Vaccine group	Vaccine name	Vaccine type	Malarial antigen targeted	Mechanism of Action
1.	Pre-erythrocytic vaccines	PfCSP vaccines		PfCSP	Antibodies to PfCSP block the sporozoite invasion of liver cells
		RTS,S/AS01 and RTS,S/AS02	Subunit vaccines	Hepatitis B surface antigen and the central repeat and C-terminal regions of CSP	Protective immune responses after vaccination with RTS,S are dependent primarily on antibody responses against the central repeat region
		PfCSP bacteria vaccines		PfCSP	Bacteria need assistance to stimulate the innate immune system
		TRAP vaccines	Subunit vaccines	SSP-2	Antibodies to SSP-2 block the invasion of mosquito salivary glands and hepatocytes
		LSA vaccines	Subunit vaccines	LSA-1/LSA-3	Elicit a pre-erythrocyte antigen response in the majority of individuals from different age groups
		PfSPZ vaccines	Live attenuated vaccines	PfSPZ	Antibodies to PfSPZ block the parasite arrival to the liver or during their development in this organ
2.	Asexual blood-stage vaccines	MSP1 vaccines	Subunit vaccines	MSP142/MSP138/MSP183	Antibodies to MSP1 to block the parasite invasion of erythrocyte
		AMA-1 vaccines	Subunit vaccines	AMA-1	AMA-1 plays an essential role in parasite survival. Antibodies to AMA-1 may kill the parasite
		Rh5 vaccines	Subunit vaccines	Rh5	Antibodies to Rh5 block the parasite invasion of erythrocyte by forming complex with cyrpa and ripr
3.	Transmission blocking vaccines	Pfs25 vaccines	Subunit vaccines	Pfs25	Pfs25 is the target on which the parasite survives and interacts with the mosquito midgut. Antibodies to Pfs25 control the transmission of malaria parasites from human hosts to the mosquito vectors
		Pfs48/45 vaccines	Subunit vaccines	Pfs48/45 C-terminus	Pfs48/45 is the target on which male gamete attaches to female gamete. Antibodies to Pfs48/45 can induce transmission-blocking antibody response during infection

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