ADVANCEMENTS IN MALARIA VACCINE: A WORK IN PROGRESS FOR ERADICATING A GLOBAL THREAT

Abstract

Malaria is a life-threatening vector-transmissible infectious disease caused by *Plasmodium* and transmitted via female *Anopheles* mosquitoes. Based on WHO (World Health Organization) nearly half of the world's population was at risk of malaria in 2021 affecting 247 million people and killing 6,19,000 of them. The disease has sustained in our society without a substantial solution for at least more than 30 years. The major difficulties to develop a vaccine In addition to the parasite's ability to elude the human immune system and the lack of sterile immunity in the human population, other factors working against malaria include the parasite's incredibly complex life cycle, biochemistry, and DNA. The high parasite burden, expense, accessibility, vaccine safety laws, and research financing are a few further obstacles to the successful development of a malaria vaccine. Based on the studies conducted till date besides designing whole parasite vaccines a lot of different *Plasmodium* antigens have been targeted for vaccine development depending upon the final efficacy of the vaccine, earliness of the stage, diversity of the antigen etc.The first Malaria vaccine approved by WHO wasRTS,S/AS01 (Mosquirix™) in the year 2021 which was developed together by GlaxoSmithKline (GSK) and the PATH Malaria Vaccine Initiative (MVI). The chapter briefly highlights the encumbrance in the path of Malaria vaccine development and the classification of the candidate vaccines with their positives and negatives. While challenges persist in vaccine design,

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production, and distribution, the progress made in recent years instills hope and optimism for a malaria-free future.

I. INTRODUCTION

Malaria is a potentially fatal infectious disease that is transmitted by vectors, primarily through the bites of female Anopheles mosquitoes carrying the *Plasmodium parasite*[1]. It is most prevalent in tropical and subtropical regions, with a significant presence in sub-Saharan Africa, as well as parts of Asia, Latin America, and the Middle East[2]. Common species of Plasmodium that infect humans include *Plasmodium vivax*, *Plasmodium malariae*, *Plasmodium falciparum*, and *Plasmodium ovale*. Among these, *P. falciparum* is responsible for the most severe and fatal cases, with a higher prevalence in Africa, while *P. vivax* is the second most common species, found in South and Central America, South and Southeast Asia, and some North African and European countries[3]. According to the World Health Organization (WHO), nearly half of the world's population was at risk of malaria in 2021, with 247 million reported cases and 619,000 deaths that year. The African region bears a disproportionate burden, accounting for 95% of malaria cases and 96% of malaria-related deaths, with approximately 80% of the affected individuals being children under the age of 5, which is truly tragic[2]. Over the years, various efforts were made to develop a malaria vaccine, but due to multiple challenges, it took a considerable amount of time before the first malaria vaccine, RTS,S/AS01, marketed as Mosquirix™ by GlaxoSmithKline (GSK) and the PATH Malaria Vaccine Initiative (MVI), received WHO approval in 2021[4].

II. WHY DID IT TAKE A LONG TIME TO DEVELOP A MALARIA VACCINE ?

Although the challenges to develop a Malaria vaccine has been known for at least more than 30 years addressing them at the forefront has been a very difficult task for the research community. The complications owe to several factors unique to the malaria parasite and the disease it causes. The main challenges were the incredibly complex life cycle, biochemistry, and genome of the parasite *P. falciparum*, as well as the parasite's ability to elude the human immune system[4,5] and lack of sterile immunity to the illness[6]. In the current section we will be delving with each of them in detail.

1. **Complex Life Cycle :** In a layman’s language the parasite *Plasmodium*completes *its life* cycle in two different hosts (mosquitoes and humans) with broadly three different *stagesnamely*- the mosquito sexual stage, the pre-erythrocytic stage and the asexual erythrocytic stage. The sporozoite form of the *Plasmodiumenter* the human blood stream when an infected Anopheles mosquito inserts its proboscis to suck blood from a healthy man. After entering the circulation, the sporozoites actively travel to the liver through the peripheral vascular system. There, they multiply in hepatocytes to produce merozoites, which are then discharged back into the bloodstream. The pre-erythrocytic stage is the phase before the merozoites are discharged into the bloodstream[7]. The erythrocytic stage begins when the merozoites begin to infect red blood cells (RBCs). After passing through the ring, trophozoite, and schizont stages, the first batch of merozoites forms new merozoites, which are released from the schizonts and infect fresh RBCs[1,8]. A tiny percentage of blood-stage (erythrocytic stage) parasites mature into sexual stage gametocytes, which are then picked up by another mosquito after passing through the dermal microvasculature[3]. Infectious sporozoites are created following fertilization and sporogonic development in the mosquito's stomach (the sexual stage of the mosquito).

These sporozoites mature in 2–14 days and enter the salivary glands where they can spread to new hosts[5].

2. **Genetic and Antigenic Diversity:** The *Plasmodium*showcases considerable genetic diversity with multiple strains and subtypes prevalent in different geographical regions. The variety of parasite populations, the dynamics of regional and temporal transmission, and the complexity of infections have all been determined using genetic markers. More specifically, the *pvcsp* gene in *Plasmodium vivax*, which produces CSP (Circumsporozoite protein), a protein considered to be the best candidate protein for vaccine development has shown to have multiple allelism due to presence of 48 polymorphic sites in parasites captured from the Brazilian Amazon and the Rio de Janeiro Atlantic Forest[9]. Similar studies on *P.vivax* populations from the China-Myanmar border where researchers using PCR-RFLP looked upon variation in surface proteins of Merozoites found three major size variants for Pvmsp-3α and four for Pvmsp-3β among the 370 and 378 samples, respectively. They concluded thatmigrant laborers from Myitsone and indigenous residents from Laiza harbored overlapping but genetically distinct *P. vivax* parasite populations[10]. But these are only two examples. As a single-celled eukaryote *Plasmodium's* complexity is far more profound than that of a bacteriumor a virus. Genome sequencing of the *Plasmodium* reveals that it can produceabout 5300 different proteins[11]. Because tiny antigens make up less than 1% of the entire parasite, the immune response spectrum elicited by current subunit malaria vaccines based on one or more protective antigens is rather restricted. Furthermore, unlike bacteria or viruses, Plasmodium not only multiplies but at specific phases develops distinctive characteristics that indicate differentiation and growth. As a result, there is clear stage specificity and significant differences between the Plasmodium antigens in each stage. As a result, a potential vaccination for one stage of Plasmodium usually cannot be used effectively against other stages. Also, developing a broad spectrum vaccine that provides protection against diverse strains has been challenging[4].

3. **Ability to swiftly evade host defenses:** Ookinetes, an advanced sexual stage in mosquito post zygote formation, secrete degradative enzymes, such as chitinase, that disintegrate the physical peritrophic membrane of the mosquito. In addition to that, ookinetes also express surface proteins such as P25, P28, and P47 that evade their digestion by mosquito's midgut proteases. The entry of sporozoites through human skin can though be encountered by neutrophils those can phagocytose them but unfortunately there are only few reports suggesting something like this significantly happens to affect the infection process[12]. Moreover, *P. falciparum* produces agaphelin which is secreted via the mosquito saliva that additionally inhibits human neutrophils' activities. Sporozoite movement through the dermis is also attributed to TRAP (thrombospondin-related anonymous protein), another sporozoite surface protein. TRAP also interacts with host cells during the pre-erythrocytic stage by binding to sulfated glycoconjugate motifs, which enables cell surface recognition and entrance into liver cells. Apart from TRAP, sporozoites need cell traversal proteins like SPECT1 (a sporozoite microneme protein required for cell traversal) and SPECT2 to successfully migrate to the liver.The consumption of hemozoin, a parasite pigment, damages monocyte and macrophage function and suppresses their capacity to release inflammatory cytokines, despite the fact that monocytes can stop the growth of parasites through antibody-dependent cellular inhibition (ADCI)[1].In the liver, the Kuffer cells (KCs) are unique phagocytic cells. The

production of ROS is a crucial immune system defensive mechanism against infection. The heparin sulfate proteoglycans found on the surface of KCs are bound by the circumsporozoite protein (CSP) in sporozoites. Moreover, LRP-1 (low-density lipoprotein-related protein) and CSP interact to increase intracellular cAMP/EPAC levels and inhibit ROS production. Moreover, many tandem repeats found in CSP decrease NF-kB signaling, which has a deleterious effect on host immune processes, in addition to downregulating antibody isotype development against it[1]. Furthermore, by the overexpression of the host heme oxygenase-1 protein (HO-1), sporozoites modify the inflammatory responses of their hosts. Furthermore, they envelop their cell surface in a parasitophorous vacuolar membrane (PVM), shielding it from apoptosis and selective autophagy. Through its surface molecule Pf92, *P. falciparum* merozoites and infected RBC at the erythrocytic stage of human infection bind to factor H (fH), a complement regulator factor, and its alternatively spliced form, fH-like protein 1. Additionally, because RBCs lack MHC molecules on their surface, CD8+ T-cells are unable to recognize erythrocytic merozoites. Gametes in the mosquito bind the fH via PfGAP50. This provides defense against complement-mediated lysis activation in both scenarios. Furthermore, Pfs47 is expressed by ookinetes, which inhibits the c-Jun N-terminal kinase pathway and stops mosquito midgut epithelium nitration, rendering the parasite immune system invisible.(5).

Note that these are only a few examples describing the immune evasion and survival mechanism of *Plasmodium* in its hosts. Further details regarding this is out of scope and hence not discussed here.

4. **Other reasons:** Few other reasons for delay in development of a successful Malaria vaccine is high parasite burden, cost, accessibility andvaccine safety, regulatory hurdles and funding.

The development of a successful malaria vaccine has been impeded by various challenges. One significant obstacle is the high parasite burden prevalent in areas with intense malaria transmission, where individuals are constantly exposed to the parasite, making it difficult to achieve long-lasting protection. Additionally, the disease's prevalence in low-resource settings with limited healthcare infrastructure presents challenges in developing a cost-effective vaccine that can be easily administered and stored[13]. Ensuring vaccine safety is of utmost importance, and past malaria vaccine candidates have faced safety concerns, necessitating rigorous testing and monitoring during clinical trials. The regulatory pathway for vaccine development can be arduous and time-consuming, as meeting the stringent requirements for safety and efficacy is crucial in gaining regulatory approval[14,15]. Furthermore, the development of vaccines demands substantial financial investment and sustained support, which has been a challenge to secure. These multifaceted challenges highlight the complexity of malaria vaccine development and the need for concerted efforts and resources to overcome them and ultimately achieve a successful vaccine that can combat this global health threat effectively.

III. SUITABLE TARGETS ATTEMPTED FOR THE DEVELOPMENT OF A MALARIA VACCINE ?

Based on the studies conducted till date besides designing whole parasite vaccines a lot of different *Plasmodium* antigens have been targeted for vaccine development depending upon the final efficacy of the vaccine, earliness of the stage, diversity of the antigen etc. Since there are a lot of stages involved during the development and infection of *P. falciparum*the target antigens are grouped based on the stage at which they occur in the parasite's life cycle. The most suitable candidate antigens in the pre-erythrocytic stage are CSP and TRAP[16]. The repeating amino acid asparagine-alanine-asparagine-proline (NANP) motifs found in the highly conserved protein domain structures of the CSP protein of P. falciparum sporozoites. It has been demonstrated that CSP causes high antibody titers, suggesting that they play a part in providing protection in animal models. When generated as a recombinant P. falciparum TRAP (PfTRAP) protein together with an adjuvant, TRAP, which is essential for sporozoite motility in one of the animal-based studies, has demonstrated that immunization with PfTRAP induced Th1 immune response and high titers of protective IgG antibodies[1]. Another target that has been pursued is the apical membrane antigen 1 (AMA1), a protein critical for merozoite invasion of human red blood cells. Preclinical studies with AMA1-based vaccines have shown promise in animal models, but clinical trials have encountered challenges in achieving high levels of protection[17]. Other merozoite surface proteins, such as MSP1 and MSP2, have also been targeted, but their efficacy has been limited in clinical trials[4,18]. Another interesting protein that has been targeted for Malaria vaccine development is RPL6. RPL6, a ribosomal protein, serves as a natural peptide antigen expressed by Plasmodium during the pre-erythrocytic stage of infection. Vaccines targeting RPL6 have demonstrated effective protection by eliciting a response from liver tissue-resident memory (TRM) cells against challenges posed by P. berghei sporozoites in mice[1]. Another promising candidate for targeting is Liver Stage Antigen-5 (LSA-5), known for its high antigenicity, with roughly 90% of individuals living in endemic areas showing antibodies against this antigen. Immunization with LSA-5 has provided protection against challenges from both P. yoelii (in mice) and P. falciparum (in Aotus monkeys). These results suggest that LSA-5 could be a crucial candidate antigen for a pre-erythrocytic subunit vaccine against malaria[3]. Additionally, genetic diversity analysis has revealed low genetic diversity and highly conserved sequences in P. vivax, leading to the identification of the vaccine candidate antigen MSP119 during the erythrocytic stage[1].

In the erythrocytic stage, the Reticulocyte-binding proteins homologous to the P. falciparum family (PfRh) that are involved in binding and initiating invasive merozoite entry into erythrocytes represent suitable vaccine targets. Ongoing studies are dedicated to uncovering more erythrocytic target antigens for malaria vaccines.

During the sexual stage, antigens such as P. falciparum 48/45 (Pfs48/45), which plays a crucial role in male gamete fertility and zygote formation, have been explored as candidate vaccine targets for malaria. Additionally, surface antigens like P. falciparum P47 (Pfs47) or P. vivax P47 (Pfs47) and P. falciparum gliding-associated protein 50 (PfGAP50) have been investigated as potential vaccine targets for the sexual stage of Plasmodium[3].

IV. DIFFERENT TYPES OF CANDIDATE MALARIA VACCINES, THEIR UPSIDES AND DOWNFALLS

The need to develop vaccine against malaria has been in discussion since 1897, close to the period when the parasite got discovered but as discussed earlier the development has been challenging till date. Below are the different types of Malaria Vaccines in development as of date.

V. BASED ON STAGES

1. **Whole sporozoite vaccines (WSV):** Whole sporozoite vaccines (WSVs) are a type of malaria vaccine that use live, weakened (attenuated) or radiation-attenuated malaria sporozoites as the vaccine antigen. Unlike subunit vaccines that use specific components of the parasite, WSVs utilize the entire sporozoite stage of the *Plasmodium* parasite to induce an immune response. This approach aims to mimic natural infection and elicit robust immune responses against multiple stages of the parasite's lifecycle.The Sanaria® PfSPZ Vaccine is a leading example of a whole sporozoite vaccine. It is a radiation-attenuated sporozoite vaccine that uses live sporozoites irradiated with gamma radiation to render them non-infectious[17]. Clinical trials have shown that this vaccine can provide high levels of protection against controlled human malaria infection (CHMI) in vaccinated individuals. The Sanaria® PfSPZ Vaccine has demonstrated promising results in both adults and children, making it one of the most advanced WSV candidates.The SPf66 vaccine was one of the earliest whole sporozoite vaccine candidates. It consisted of a mixture of synthetic peptides derived from different sporozoite surface antigens. However, despite initial hopes, clinical trials showed limited efficacy, and the vaccine failed to provide significant protection against malaria. Consequently, the SPf66 vaccine was not widely adopted for malaria control efforts[19,20].The PfCS102 vaccine was based on the circumsporozoite protein (CSP) of the *Plasmodium falciparum* parasite. It used recombinant CSP combined with an adjuvant to boost the immune response. While initial preclinical studies showed promise, subsequent phase II clinical trials in Africa demonstrated insufficient protective efficacy, leading to discontinuation of further development[21].

2. **TBV (Transmission Blocking Vaccines):** Transmission-blocking vaccines (TBVs) are a unique type of malaria vaccine designed to interrupt the transmission of the malaria parasite from humans to mosquitoes. Unlike traditional vaccines that primarily aim to protect the vaccinated individual from clinical disease, TBVs target antigens expressed on the sexual stages (gametocytes) of the Plasmodium parasite, which are responsible for infecting mosquitoes during a blood meal. By targeting these sexual stages, TBVs aim to prevent the transmission of malaria from human hosts to mosquito vectors, thus interrupting the malaria transmission cycle[17].The Pfs25 and Pfs28 proteins are important targets for TBVs as they are essential for the sexual development of the malaria parasite in mosquitoes[3]. Several experimental vaccines based on these antigens have shown promising results in animal studies and early-phase clinical trials. These vaccines elicited antibodies in vaccinated individuals that effectively blocked the development of the parasite in mosquitoes, reducing the potential for onward transmission.Pfs230 is another crucial antigen involved in the sexual development of the malaria parasite in mosquitoes. Several attempts have been made to develop vaccines based on Pfs230, but

achieving a robust immune response against this antigen has proven challenging. Some early clinical trials of Pfs230-based vaccines did not show the desired level of transmission-blocking activity, highlighting the need for further optimization and research.Some TBV candidates have been evaluated in combination with other vaccine types, such as pre-erythrocytic or blood-stage vaccines, to achieve a more comprehensive immune response. While combining different vaccine approaches may have potential benefits, it also introduces additional complexities and challenges in vaccine development, which might impact the overall success of TBVs[4].

3. **Pre-erythrocytic vaccines (PEV):** Pre-erythrocytic vaccines (PEVs) are a category of malaria vaccines that target the early stages of the malaria parasite's lifecycle, specifically before the parasite infects red blood cells. The objective of PEVs is to prevent the infection from progressing to the symptomatic blood stage, where the majority of clinical malaria symptoms occur. These vaccines aim to induce strong immune responses against the sporozoite and liver stages of the *Plasmodium* parasite, ultimately blocking its development and replication within the human host[4].The RTS,S/AS01 vaccine is one of the most well-known and advanced pre-erythrocytic vaccines. It targets the circumsporozoite protein (CSP) on the surface of sporozoites and uses the AS01 adjuvant system to enhance the immune response. The RTS,S/AS01 vaccine has undergone extensive clinical testing, including large-scale phase III trials in several African countries. The vaccine demonstrated partial protection against clinical and severe malaria in young children and infants, leading to its approval by the World Health Organization (WHO) for use in selected areas with moderate to high malaria transmission[17].The PfSPZ-CVac vaccine, developed by Sanaria®, is another pre-erythrocytic vaccine candidate that uses radiation-attenuated sporozoites. Despite showing promise in early-phase clinical trials, a phase IIb trial conducted in Equatorial Guinea did not meet the primary endpoint of preventing malaria infection. The vaccine showed limited efficacy in the trial population, highlighting the challenges in achieving high levels of protection and the need for further research and optimization[1,17].

4. **Blood-stage vaccines (BSV):** Blood-stage vaccines (BSVs) are a class of malaria vaccines that target the asexual stages of the malaria parasite's lifecycle, particularly the merozoite and trophozoite stages that circulate in the bloodstream. Unlike pre-erythrocytic vaccines that aim to prevent the infection from progressing to the blood stage, BSVs focus on inducing immune responses that target and eliminate the parasite during the blood stage, thereby reducing the severity of clinical symptoms and preventing severe malaria outcomes[4].The MSP1 is one of the major antigens expressed on the surface of the merozoite stage of the *Plasmodium* parasite. Several blood-stage vaccines targeting MSP1 have been developed and tested in clinical trials. Some of these vaccines have shown promising results in inducing immune responses and reducing the severity of malaria symptoms. Although no MSP1 vaccine has yet reached widespread implementation, their success in early-phase trials suggests their potential as components of future multi-stage malaria vaccines[1].AMA1 is another important antigen expressed on the surface of the merozoite stage of the malaria parasite. Several AMA1-based blood-stage vaccines have been evaluated in clinical trials. While some of these vaccines showed promising results in preclinical studies, the efficacy in clinical trials was not sufficient to progress to wide-scale implementation. Challenges with antigen diversity and

antigenic variation in different malaria strains have complicated the development of effective AMA1 vaccines[18,19,22].

5. **Multi-stage Malaria Vaccine:** A multi-stage malaria vaccine is a type of vaccine that targets multiple stages of the malaria parasite's lifecycle, aiming to provide comprehensive protection against the disease. It combines antigens from different stages of the parasite, including pre-erythrocytic (sporozoite and liver stages) and blood-stage antigens (merozoite and trophozoite stages), to induce immune responses that can prevent infection, reduce parasite burden, and alleviate clinical symptoms[23].One example of a multi-stage malaria vaccine candidate is the ME-TRAP vaccine. ME-TRAP is a combination vaccine that includes both pre-erythrocytic and blood-stage antigens. It combines the thrombospondin-related adhesion protein (TRAP) from the sporozoite stage with multiple epitopes (short fragments of proteins) from the liver and blood stages of *Plasmodium falciparum*. The ME-TRAP vaccine has shown promising results in preclinical studies and early-phase clinical trials, inducing immune responses against multiple stages of the malaria parasite[24].The FMP2.1/AS02A vaccine is an example of a multi-stage malaria vaccine that did not achieve the desired level of success. It was a combination vaccine that included the merozoite surface protein 2.1 (MSP2.1) from the blood stage and the apical membrane antigen 1 (AMA1) from the sporozoite and blood stages of *Plasmodium falciparum*. Despite promising preclinical data, a phase IIb clinical trial conducted in African children did not show sufficient protective efficacy against clinical malaria, leading to the discontinuation of further development[20].

VI. BASED ON DEVELOPMENTAL STRATEGY

1. **Subunit Vaccines:** Subunit vaccines contain specific components of the malaria parasite, such as proteins or peptides, that elicit an immune response without causing the disease. These components are selected based on their ability to induce a protective immune response. Examples include vaccines targeting the circumsporozoite protein (CSP) or merozoite surface proteins (MSPs).RTS,S/AS01 is a subunit malaria vaccine that contains a portion of the CSP antigen.The vaccine aims to induce an immune response against the CSP protein present on the surface of the sporozoite stage of the malaria parasite. By targeting this stage, RTS,S/AS01 aims to prevent the parasite from establishing infection in the liver and progressing to the blood stage, thereby reducing the incidence of clinical malaria[4].MSP1 is one of the major surface proteins of the merozoite stage of the malaria parasite. Several vaccine candidates have targeted this antigen to induce immune responses that can block merozoite invasion of red blood cells. The MSP1 subunit vaccine aims to elicit antibodies that can neutralize merozoites and prevent them from infecting red blood cells, thus reducing the parasite burden and clinical symptoms of malaria.[25,26].

2. **Whole-Parasite Vaccines (WSVs):** Whole-parasite vaccines use live, weakened (attenuated) parasites or killed (inactivated) parasites as the vaccine antigen. These vaccines aim to stimulate both cellular and antibody-mediated immunity against multiple parasite stages, including the sporozoite and liver stages. Whole-organism vaccines have shown promise in clinical trials, particularly in providing protection against the liver stage of the parasite[17]. The examples for WSVs are already mentioned above.

3. **Vectored Vaccines:** Vectored vaccines use non-pathogenic viruses or bacteria (vectors) to deliver genes encoding specific malaria antigens into the human body. These antigens are then expressed, leading to the production of the target proteins by the host's cells. Viral vectors, such as adenoviruses and poxviruses, are commonly used in vectored vaccine platforms. For example, The ChAd63-MVA ME-TRAP vaccine uses a two-step viral vector approach. First, a chimpanzee adenovirus vector, ChAd63, is used to deliver the ME-TRAP antigen into the cells. Then, a modified vaccinia virus Ankara, MVA, is used as a booster dose to enhance the immune response. ME-TRAP is a fusion protein containing multiple epitopes of the *Plasmodium falciparum* thrombospondin-related adhesion protein, which is present on sporozoites. This vaccine aims to induce both cellular and humoral immune responses against sporozoites to prevent infection in the liver. Similarly, AdCh63 MSP1 is a viral vector vaccine that uses a chimpanzee adenovirus vector, AdCh63, to deliver the MSP1 antigen. MSP1 is a key antigen expressed on the surface of the merozoite stage of the malaria parasite. By delivering the MSP1 antigen using the viral vector, the vaccine aims to elicit a robust immune response that can block merozoite invasion of red blood cells and reduce the parasite burden.AdCh63 AMA1 is a viral vector vaccine that uses the AdCh63 vector to deliver the AMA1 antigen. AMA1 is an important antigen involved in merozoite invasion of red blood cells. The vaccine aims to induce a strong immune response against AMA1 to block merozoite entry into red blood cells and reduce the severity of malaria.AdHu5-MSP1 is a viral vector vaccine that uses a human adenovirus 5 vector to deliver the MSP1 antigen. Similar to AdCh63 MSP1, this vaccine aims to elicit an immune response against MSP1 to block merozoite invasion and reduce the parasite burden[23,27].

4. **DNA Vaccines:** DNA vaccines involve the direct injection of plasmid DNA encoding malaria antigens into the host. The host's cells then use the DNA to produce the antigen proteins, triggering an immune response. DNA vaccines have the advantage of being relatively easy to produce and can be tailored to target multiple antigens.The PfSPZ DNA vaccine is a DNA-based vaccine that uses the genetic material encoding the sporozoites of Plasmodium falciparum, the malaria parasite. This vaccine aims to stimulate an immune response against the sporozoites to prevent their invasion of the liver cells, thereby blocking the early stage of malaria infection[28].

5. **Transmission-Blocking Vaccines:** Transmission-blocking vaccines are designed to focus on antigens expressed during the sexual stage, known as gametocytes, with the objective of inhibiting transmission of the parasite from humans to mosquitoes. It's important to note that these vaccines do not provide protection against clinical disease for individuals but play a significant role in supporting malaria control and elimination initiatives. Detailed examples of such vaccines are provided in the preceding section.

VII. ABOUT THE WHO APPROVED FIRST MALARIA VACCINE RTS,S/AS01

The RTS,S/AS01 malaria vaccine, also recognized as Mosquirix™, holds the distinction of being the first and presently the sole vaccine endorsed by the World Health Organization (WHO) for malaria prevention. This achievement is a pivotal moment in the battle against this deadly disease that impacts millions of individuals, particularly in sub-Saharan Africa. The collaborative effort of GlaxoSmithKline (GSK) and the PATH Malaria Vaccine Initiative (MVI) led to the vaccine's development. In the ensuing discussion, we will

delve into the vaccine's origins, clinical trials, effectiveness, implementation, and the obstacles it has encountered.

The development journey of the RTS,S/AS01 vaccine commenced in the late 1980s when GSK scientists pinpointed the circumsporozoite protein (CSP) as a promising target for malaria vaccine research. CSP is a predominant surface protein found on the sporozoite stage of the Plasmodium falciparum parasite, which is transmitted to humans via the bites of infected mosquitoes. Recognizing the substantial malaria burden in sub-Saharan Africa, GSK partnered with the MVI to advance the vaccine's development.

The RTS,S/AS01 vaccine is a subunit vaccine that combines the CSP antigen with the hepatitis B surface antigen (HBsAg). The role of HBsAg is to serve as a carrier protein, enhancing the immune response to the CSP antigen. Furthermore, the vaccine incorporates the AS01 adjuvant system, developed by GSK, to further enhance the immune response. This adjuvant includes QS-21, an immunostimulatory compound derived from the bark of the Quillajasaponaria tree, and liposomes, which improve the presentation of antigens to the immune system[29,30].

The clinical development of the RTS,S/AS01 vaccine involved a comprehensive series of phase I, II, and III clinical trials conducted over an extended period. These trials were designed to assess the vaccine's safety, its ability to generate an immune response, and its effectiveness in diverse populations, including children and infants living in malaria-endemic regions. Phase I and II trials demonstrated that the RTS,S/AS01 vaccine was well-tolerated and elicited strong immune responses against the CSP antigen. These early trials played a pivotal role in refining the vaccine's formulation and dosing.

The pivotal phase III trials of the RTS,S/AS01 vaccine, conducted under the umbrella of the RTS,S Clinical Trials Partnership (CTP), took place at multiple sites across sub-Saharan Africa, where malaria is prevalent. These trials included thousands of children and infants at risk of malaria. The first phase III trial, carried out from 2009 to 2014 in seven African countries, assessed the vaccine's effectiveness against clinical malaria in children aged 5-17 months. The study revealed that the vaccine reduced the risk of clinical malaria by approximately 40% and severe malaria by 30% over a four-year follow-up period. The second phase III trial, conducted from 2012 to 2014, evaluated the vaccine's efficacy in younger infants (6-12 weeks old) and explored the potential for booster doses.

This study demonstrated that the vaccine exhibited reduced efficacy in younger infants but still offered a degree of protection against clinical malaria. In 2015, the World Health Organization (WHO) advised the pilot implementation of the RTS,S/AS01 vaccine in multiple African countries to assess its practicality, impact, and safety in real-world scenarios. In 2019, following further data analysis and evaluation, the WHO endorsed the inclusion of the vaccine in routine childhood immunization programs in selected areas characterized by moderate to high malaria transmission rates.

In October 2021, the WHO officially granted approval for the use of the RTS,S/AS01 malaria vaccine in children aged 5 months to 2 years. This approval represents a significant advancement in malaria control efforts and paves the way for wider implementation and impact[4]. The RTS,S/AS01 vaccine has demonstrated noteworthy effectiveness in lowering

the risk of clinical and severe malaria, especially in children aged 5-17 months. Across the four-year follow-up period of the phase III trials, the vaccine delivered partial protection against malaria, a notable accomplishment given the intricacies of malaria immunity.

Introducing the RTS,S/AS01 vaccine into real-world settings presents several challenges. A primary concern is the necessity for a four-dose schedule, potentially posing difficulties in ensuring that children receive all doses on time. Moreover, maintaining the cold chain for vaccine distribution and storage in resource-limited settings can be demanding, as the vaccine requires specific temperature conditions for stability. This underscores the need for a robust healthcare infrastructure and comprehensive training for healthcare personnel.

Despite its partial efficacy, the vaccine holds the potential to prevent millions of malaria cases and save thousands of lives, particularly in areas with moderate to high malaria transmission. By alleviating the disease burden, the vaccine can also contribute to improved childhood health, educational outcomes, and economic development in malaria-endemic regions. The approval and endorsement of the RTS,S/AS01 vaccine signify a significant milestone in the development of malaria vaccines[3,4,20,31,32].

For more comprehensive protection against malaria, however, research is still being conducted to enhance vaccine efficacy and investigate alternative targets and vaccine candidates.Novel vaccination technologies, like DNA vaccines, vectored vaccines, and nanoparticle-based techniques, are being studied by researchers in an effort to boost immune responses and possibly increase vaccine efficacy. Furthermore, the discovery of novel target antigens and enhanced adjuvants could result in the creation of malaria vaccines of the next generation that offer more comprehensive defense against a wider range of parasite strains.

VIII. CONCLUSIONS

In 2021 Malaria stood out to be one of the most dangerous vector-transmissible infectious diseases.The attempts to develop vaccine against malaria started as early as 1897 but due to several complications such as complex life cycle, biology and genome of the *Plasmodium* it has been difficult to develop an effective vaccine. Albeit all the difficulties, the technology for development of the Malaria vaccine has advanced greatly. There has been a lot of studies and innovations that will tread the roadmap for future discoveries in the field of vaccine development. The approval of the first malaria vaccine RTS,S/AS01 as Mosquirix™ by WHO in the year 2021 is though a milestone the maximum efficacy of it is not more than 50 per cent. While challenges persist in vaccine design, production, and distribution, the progress made in recent years instills hope and optimism for a malaria-free future. Continued collaboration, research, and investment are essential to bring these promising vaccine candidates to fruition, safeguarding millions of lives and paving the way towards eradicating malaria as a global health burden.

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