**Advancements in Malaria Vaccine: A Work in Progress for Eradicating a Global Threat**

**Introduction**

Malaria is a life-threatening vector-transmissible infectious disease caused by *Plasmodium* and primarily transmitted via bites of infected female Anopheles mosquitoes. It is prevalent in tropical and subtropical regions, particularly in sub-Saharan Africa, but also occurs in parts of Asia, Latin America, and the Middle East. *Plasmodium vivax*, *Plasmodium falciparum*, *Plasmodium malariae*, and *Plasmodium ovale* are among the species that commonly affect humans. *P. falciparum* is responsible for the most severe forms of disease and death and is more prevalent in Africa. The second most common species is *P. vivax*, found in South and Southeast Asia, Central and South America, and some countries in Europe and North Africa. Based on WHO (World Health Organization) nearly half of the world's population was at risk of malaria in 2021. The disease affected 247 million people killing 6,19,000 of them as reported the same year. The African region carries a disproportionally high share of the global malaria burden. In 2021, the region housed 95% of malaria cases and 96% of malaria deaths. Among them children < 5 years accounted for about 80% which is heart breaking. In the past, there has been several attempts to design a vaccine against malaria but due to several miscellaneous factors it took a very long time to finally extradite the first malaria vaccine RTS,S/AS01 as Mosquirix™ by GlaxoSmithKline (GSK) and the PATH Malaria Vaccine Initiative (MVI) together that got approved by WHO in the year 2021.

**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 difficulties were the parasite’s (*P. falciparum*) extremely complex life cycle, biology and genome in addition to the parasite’s evasion from the human immune system and the absence of sterile immunity to the disease. 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 itslife cycle in two different hosts (mosquitoes and humans) with broadly three different stages namely- the mosquito sexual stage, the pre-erythrocytic stage and the asexual erythrocytic stage. The sporozoite form of the Plasmodium enter the human blood stream when an infected Anopheles mosquito inserts its proboscis to suck blood from a healthy man. Once the sporozoites enter the blood stream they actively reach the peripheral vascular system and migrate to the liver where they replicate in hepatocytes, forming merozoites that are released into the bloodstream. The step till the merozoites are released into the blood stream is called the pre-erythrocytic stage. Once the merozoites start infecting the red blood cells (RBCs) the erythrocytic stage starts. The first batch of merozoites then develop through the ring, trophozoite, and schizont stages before forming new merozoites that are released at the exit of the schizonts and reinfect new RBCs. A small number of the blood-stage (erythrocytic stage) parasites develop into sexual stage gametocytes, which pass through microvasculature in the dermis and are captured by another mosquito. After fertilization and sporogonic development in the gut of the mosquito (the mosquito sexual stage), infectious sporozoites are formed those mature in 2-14 days and reach the salivary glands to be transmitted to another host.

2. Genetic and Antigenic Diversity: The *Plasmodium* showcases considerable genetic diversity with multiple strains and subtypes prevalent in different geographical regions. Genetic markers have been used to determine the diversity of parasite populations, spatial and temporal transmission dynamics, and complexity of infections. 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. 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 that migrant laborers from Myitsone and indigenous residents from Laiza harbored overlapping but genetically distinct *P. vivax* parasite populations. But these are only two examples. As a single-celled eukaryote *Plasmodium’s* complexity is far more profound than that of a bacterium or a virus. Genome sequencing of the *Plasmodium* reveals that it can produce about 5300 different proteins. Therefore, the immune response spectrum induced by existing subunit malaria vaccines based on one or several protective antigens is relatively narrow as small antigens represent less than 1% of the whole parasite. Moreover, unlike a bacterium or a virus, *Plasmodium* not only proliferates but also acquires characteristic features signifying differentiation and development at certain stages. Thus, the antigens of Plasmodium in each stage are quite different, and there is obvious stage specificity. Hence, a candidate vaccine against a certain stage of *Plasmodium* generally cannot play an effective role in other stages. Also, developing a broad spectrum vaccine that provides protection against diverse strains has been challenging.

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. Moreover, *P. falciparum* produces agaphelin which is secreted via the mosquito saliva that additionally inhibits human neutrophils’ activities. Another sporozoite surface protein, TRAP (thrombospondin-related anonymous protein) is also responsible for sporozoite motility through the dermis. In the pre-erythrocytic stage TRAP also interacts with host cells through binding to sulfated glycoconjugates motifs which results in cell surface recognition and entry to liver cells. Other than TRAP Cell traversal proteins such as SPECT1 (sporozoite microneme protein essential for cell traversal) and SPECT2 are utilized by sporozoites to achieve successful migration to the liver. Although Monocytes can inhibit the growth of parasites by antibody-dependent cellular inhibition (ADCI) ingestion of hemozoin (parasite pigment) impairs the function of monocytes and macrophages and represses their ability to produce inflammatory cytokines. The Kuffer cells (KCs) are special phagocytic cells in liver. ROS generation is an essential defense strategy in human immune system to encounter infection. The Circumsporozoite protein (CSP) in sporozoites binds to heparin sulfate proteoglycans present on the surface of KCs. CSP also interacts with LRP-1 (low-density lipoprotein-related protein), which upregulates the intracellular levels of cAMP/EPAC and prevents ROS formation. Further CSP not only has multiple tandem repeats which can downregulate antibody isotype maturation against it but also causes suppression of the NF-kB signalling which negatively affects the host immune mechanisms. Additionally ,Sporozoites alter host inflammatory responses via upregulation of host heme oxygenase-1 protein (HO-1) and form a parasitophorous vacuolar membrane (PVM) around their cell surface which protects them from selective autophagy and apoptosis. *P. falciparum* merozoites and infected RBC in the erythrocytic stage in humans, bind to the factor H (fH), a complement regulator factor, and its alternatively spliced form fH-like protein 1 through its surface molecule Pf92. Also, RBCs do not express MHC molecules on their surface and thus erythrocytic merozoites escape recognition by CD8+ T-cells. In the mosquito, gametes bind the fH through PfGAP50. In both situations, this allows protection against the activation of complement-mediated lysis. In addition, ookinetes express Pfs47, which disrupts the c-Jun N-terminal kinase pathway and prevents mosquito midgut epithelial nitration, making the parasite undetectable by complement system.

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 and vaccine 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. 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. 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.

**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. CSP protein of *P. falciparum* sporozoites contains highly conserved protein domains structures which have been characterized by repeating amino acid, asparagine-alanine-asparagine-proline (NANP) motifs. CSP has been shown to induce high antibody titers indicating their role in conferring protection in animal models. TRAP, which is critical for sporozoite motility in one of the animal-based study when produced as a recombinant *P. falciparum* TRAP (PfTRAP) protein along with an adjuvant has shown that vaccination with PfTRAP induced Th1 immune response and high titers of protective IgG antibodies. 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. Other merozoite surface proteins, such as MSP1 and MSP2, have also been targeted, but their efficacy has been limited in clinical trials. Another interesting protein that has been targeted for Malaria vaccine development is RPL6. Ribosomal protein RPL6 is a natural peptide antigen which is expressed by *Plasmodium* during pre-erythrocytic stage infection. RPL6 targeting vaccines have shown effective protection by inducing liver Tissue-resident memory (TRM) cell response against *P. berghei* sporozoites challenge in mice. Liver Stage Antigen-5 (LSA-5) is another candidate target which is highly antigenic and the prevalence of antibodies in individuals against this antigen living in endemic areas is extremely high (roughly 90%). Immunization with LSA-5 induced protection against both challenges of P. yoelii (in mice) and P. falciparum (in Aotus monkeys). The results suggest that LSA-5 could be an important antigen candidate for an anti-malaria subunit pre-erythrocytic vaccine. Based on genetic diversity analysis, low genetic diversity and highly conserved sequences have been reported in *P. vivax* leading to vaccine candidate antigen MSP119. In erythrocytic stage, Reticulocyte-binding proteins homologous of *P. falciparum* family (PfRh) which are involved in binding and initiating invasive merozoite entry into erythrocytes are suitable vaccine targets. Further studies are in continuation to discover more erythrocytic target antigens for malaria vaccine. In sexual stage, antigens like *P. falciparum* 48/45 (Pfs48/45) which plays a key role in male gamete fertility and zygote formation has been used as a candidate malaria vaccine target. Besides that, *P. falciparum* P47 (Pfs47) or *P. vivax* P47 (Pfs47) surface antigens and *P. falciparum* gliding-associated protein 50 (PfGAP50) have also been tried as candidate malaria vaccine targets in sexual stage of *Plasmodium*.

**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.

BASED ON STAGES

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. 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. 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. The VMP001 vaccine was developed by GSK and used a viral vector to deliver Plasmodium antigens, including CSP and apical membrane antigen 1 (AMA1). Despite showing some protective efficacy in early trials, a large-scale phase III trial conducted in Africa did not demonstrate sufficient efficacy in protecting against clinical malaria, leading to the discontinuation of the vaccine's development.

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. The Pfs25 and Pfs28 proteins are important targets for TBVs as they are essential for the sexual development of the malaria parasite in mosquitoes. 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.

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. 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. 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.

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. 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. 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.

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. 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. 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.

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. 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. RAP1 is an antigen that associates with the Rh5 protein on the surface of the merozoite stage. The Rh5-RAP1 complex plays a critical role in the invasion of red blood cells by merozoites. Vaccines targeting RAP1 aim to elicit antibodies that can disrupt the Rh5-RAP1 interaction, thereby preventing merozoite invasion and reducing parasite replication.

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. 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.

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.

5. Transmission-Blocking Vaccines: Transmission-blocking vaccines target antigens expressed on the sexual stage (gametocytes) of the parasite, aiming to prevent transmission from human to mosquito. These vaccines do not protect individuals from clinical disease but contribute to malaria control and elimination efforts. Examples are discussed in the previous section.

**About the WHO approved first Malaria vaccine RTS,S/AS01**

The RTS,S/AS01 malaria vaccine, also known as Mosquirix™, is the world's first and currently the only vaccine approved by the World Health Organization (WHO) for the prevention of malaria. It represents a significant milestone in the fight against this deadly disease that affects millions of people, particularly in sub-Saharan Africa. The vaccine was developed through a collaboration between GlaxoSmithKline (GSK) and the PATH Malaria Vaccine Initiative (MVI). In this detailed discussion, we will explore the development, clinical trials, efficacy, implementation, and challenges surrounding the RTS,S/AS01 malaria vaccine. The development of the RTS,S/AS01 vaccine began in the late 1980s when scientists at GSK identified the circumsporozoite protein (CSP) as a potential target for malaria vaccine development. CSP is a major surface protein on the sporozoite stage of the Plasmodium falciparum parasite, which is transmitted to humans through the bite of infected mosquitoes. GSK collaborated with the MVI to advance the vaccine's development, given the significant burden of malaria in sub-Saharan Africa.

The RTS,S/AS01 vaccine is a subunit vaccine that combines the CSP antigen with the hepatitis B surface antigen (HBsAg). The HBsAg acts as a carrier protein, enhancing the immune response to the CSP antigen. Additionally, the AS01 adjuvant system, developed by GSK, is incorporated into the vaccine to further boost the immune response. The adjuvant contains QS-21, an immunostimulatory compound derived from the bark of the *Quillaja saponaria* tree, and liposomes, which enhance antigen presentation to the immune system. The clinical development of the RTS,S/AS01 vaccine involved a series of phase I, II, and III clinical trials conducted over several years. These trials aimed to evaluate the vaccine's safety, immunogenicity, and efficacy in different populations, including children and infants in malaria-endemic regions. Phase I and II trials demonstrated that the RTS,S/AS01 vaccine was well-tolerated and induced robust immune responses against the CSP antigen. These early trials helped refine the vaccine's formulation and dosing. The pivotal phase III trials of the RTS,S/AS01 vaccine, known as the RTS,S Clinical Trials Partnership (CTP), were conducted in multiple sites across sub-Saharan Africa, where malaria is endemic. These trials involved thousands of children and infants at risk of malaria. The first phase III trial, conducted in seven African countries from 2009 to 2014, assessed the vaccine's efficacy against clinical malaria in children aged 5-17 months. The study demonstrated that the vaccine reduced the risk of clinical malaria by approximately 40% and severe malaria by 30% over four years of follow-up. The second phase III trial, conducted in 2012-2014, evaluated the vaccine's efficacy in younger infants (6-12 weeks old) and assessed the potential for booster doses. This study showed that the vaccine was less effective in younger infants but provided some protection against clinical malaria. In 2015, the WHO recommended pilot implementation of the RTS,S/AS01 vaccine in several African countries to assess its feasibility, impact, and safety in real-world settings. In 2019, after further data analysis and review, the WHO recommended the use of the vaccine as part of routine childhood immunization programs in selected areas with moderate to high malaria transmission. In October 2021, the WHO officially approved the RTS,S/AS01 malaria vaccine for use in children aged 5 months to 2 years. This approval represents a significant step in malaria control efforts and opens the door for broader implementation and impact.

The RTS,S/AS01 vaccine has shown significant efficacy in reducing the risk of clinical and severe malaria, particularly in children aged 5-17 months. Over the four-year follow-up period of the phase III trials, the vaccine provided partial protection against malaria, which is considered a meaningful achievement given the complexities of malaria immunity. The implementation of the RTS,S/AS01 vaccine in real-world settings poses several challenges. One significant concern is the need for a four-dose schedule, which may present challenges in ensuring that children receive all doses on schedule. Additionally, maintaining the cold chain during vaccine distribution and storage in resource-limited settings can be challenging, as the vaccine requires specific temperature conditions for stability. This necessitates a strong healthcare infrastructure and adequate training for healthcare workers. Despite its partial efficacy, the vaccine has the potential to prevent millions of malaria cases and save thousands of lives, especially in areas with moderate to high malaria transmission. By reducing the burden of disease, the vaccine can also contribute to improved childhood health, educational outcomes, and economic development in malaria-endemic regions. The approval and recommendation of the RTS,S/AS01 vaccine mark an essential milestone in malaria vaccine development. However, ongoing research aims to improve vaccine efficacy and explore other targets and vaccine candidates for broader protection against malaria. Researchers are investigating novel vaccine technologies, such as vectored vaccines, DNA vaccines, and nanoparticle-based approaches, to enhance immune responses and potentially achieve higher efficacy. Additionally, the identification of new target antigens and improved adjuvants may lead to the development of next-generation malaria vaccines with broader protection against diverse parasite strains.

**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.

**References**

[1] R.M. da S. Mariano, A.A.M. Gonçalves, D.S. de Oliveira, H.S. Ribeiro, D.F.S. Pereira, I.S. Santos, D.F. Lair, A.V. da Silva, A.S. Galdino, M.A. Chávez-Fumagalli, D. da Silveira-Lemos, W.O. Dutra, R.C. Giunchetti, A Review of Major Patents on Potential Malaria Vaccine Targets, Pathogens. 12 (2023). https://doi.org/10.3390/pathogens12020247.

[2] P. Chandley, R. Ranjan, S. Kumar, S. Rohatgi, Host-parasite interactions during Plasmodium infection: Implications for immunotherapies, Front. Immunol. 13 (2023) 1–27. https://doi.org/10.3389/fimmu.2022.1091961.

[3] C.A. Ezema, I.U. Okagu, T.P.C. Ezeorba, Escaping the enemy’s bullets: an update on how malaria parasites evade host immune response, Parasitol. Res. (2023) 1715–1731. https://doi.org/10.1007/s00436-023-07868-6.

[4] L. Rénia, Y.S. Goh, Malaria parasites: The great escape, Front. Immunol. 7 (2016) 1–14. https://doi.org/10.3389/fimmu.2016.00463.

[5] P.K. Kiyuka, S. Meri, A. Khattab, Complement in malaria: immune evasion strategies and role in protective immunity, FEBS Lett. 594 (2020) 2502–2517. https://doi.org/10.1002/1873-3468.13772.

[6] A. Sakoguchi, H. Arase, Mechanisms for Host Immune Evasion Mediated by Plasmodium falciparum-Infected Erythrocyte Surface Antigens, Front. Immunol. 13 (2022) 1–8. https://doi.org/10.3389/fimmu.2022.901864.

[7] E.B. Belachew, Immune Response and Evasion Mechanisms of Plasmodium falciparum Parasites, J. Immunol. Res. 2018 (2018). https://doi.org/10.1155/2018/6529681.

[8] J.H. Han, J.S. Cho, J.J.Y. Ong, J.H. Park, M.H. Nyunt, E. Sutanto, H. Trimarsanto, B. Petros, A. Aseffa, S. Getachew, K. Sriprawat, N.M. Anstey, M.J. Grigg, B.E. Barber, T. William, G. Qi, Y. Liu, R.D. Pearson, S. Auburn, R.N. Price, F. Nosten, L. Rénia, B. Russell, E.T. Han, Genetic diversity and neutral selection in Plasmodium vivax erythrocyte binding protein correlates with patient antigenicity, PLoS Negl. Trop. Dis. 14 (2020) 1–16. https://doi.org/10.1371/journal.pntd.0008202.

[9] N.K. Almeida-De-Oliveira, R. de Abreu-Fernandes, L. Lima-Cury, A.R. de Lavigne, A. de Pina-Costa, D. de Souza Perce-Da- Silva, M. Catanho, A.D. Rossi, P. Brasil, C.T. Daniel-Ribeiro, M. De Fátima Ferreira-Da-Cruz, Balancing selection and high genetic diversity of Plasmodium vivax circumsporozoite central region in parasites from Brazilian Amazon and Rio de Janeiro Atlantic Forest, PLoS One. 15 (2020) 1–20. https://doi.org/10.1371/journal.pone.0241426.

[10] X. Wang, Y. Bai, Z. Xiang, W. Zeng, Y. Wu, H. Zhao, W. Zhao, X. Chen, M. Duan, X. Li, W. Zhu, K. Sun, Y. Wu, Y. Zhang, X. Li, B.M. Rosenthal, L. Cui, Z. Yang, Genetic diversity of Plasmodium vivax populations from the China–Myanmar border identified by genotyping merozoite surface protein markers, Trop. Med. Health. 51 (2023). https://doi.org/10.1186/s41182-022-00492-7.

[11] A.G. Bashir, C. Wanna, N.-B. Kesara, Genetic diversity of pvcsp and pvs25 in Plasmodium vivax isolates in malaria-endemic areas in Asia, Africa, and America: A systematic review, African J. Pharm. Pharmacol. 17 (2023) 73–84. https://doi.org/10.5897/ajpp2023.5355.