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Malaria Vaccine RTS,S

Research Project
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ABSTRACT

Humanity has known about malaria for thousands of years as a potentially fatal disease called malaria was introduced to humans by human *Plasmodium* parasite transmission by the *Anopheles* mosquito. Africa bears the greatest burden of malaria with more than 200 million clinical cases and more than 600,000 deaths in 2020 alone. While malaria-associated deaths dropped steadily until 2015, the decline started to falter after 2016, highlighting the need for novel potent tools in the fight against malaria. In the region, 80% of all malaria deaths were in children under the age of five .Drug resistance and insecticide-resistant invertebrate vectors have complicated efforts to control this illness, despite measures in place. The development of an effective malaria vaccine would have a significant impact on the eradication and management of this disease the Researches have focused on antigens of high importance for the survival of the parasite such as the Circumsporozoite Surface Protein (CSP), involved in the pre-erythrocytic cycle during parasites invasion in hepatocytes. Currently, RTS'S is the most promising vaccine for malaria and was constructed using CSP and RTS,S/AS01 is highly immunogenic, inducing high level of anti-CSP antibodies and CSP-specific CD4 T-call responses in vaccinated individuals The CD4 T-cell responses may provide additional protection, independent of the anti-CSP antibody response. One year after the third dose of RTS,S/AS01, the effectiveness of protection against the parasite was observed in 55.8% of infant aged five to 17 month and 31.3 %of infant aged six to 12 week in 2015, the European Medicines Agency found that the RTS,S/AS01 vaccine has an acceptable safety profile that would continue to be monitored and recently (2021) RTS,S/AS01 vaccine was recommended by World health organization (WHO).

Keyword: malaria, *Plasmodium*, vaccine, pre-erythrocytic, RTS,S/AS01.

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1. INTRUDUCTION

In many parts of the world malaria continues to be serious public health issue and very common in countries that in the tropical and subtropical. Malaria remains a disease of global health importance with billion people in 97 countries at risk, leading to an estimated 200 million cases and around 600,000 deaths (WHO, 2015). This disease is brought on by the protozoan genus Plasmodium (mainly *P. falciparum*, *P. vivax*, *P. ovale* and *P. malariae*). The life cycle of the protozoa is split between a human host and an insect vector, specifically the *Anopheles* spp. mosquito. Of the 380 species of *Anopheles* genus mosquitoes, only 60 species' females may spread malaria (Heyneman1995). Despite efforts to control and prevent the disease, 228 million cases and 405 thousand fatalities were reported globally in 2018. In Africa, *Plasmodium falciparum* was the cause of 99.7% of reported cases of malaria, as well as the majority of cases in Southeast Asia (50%) and the Eastern Mediterranean (71%), Western Pacific (65%), and Southeast Asia (Geneva WHO 2019). Therefore, research has been conducted to develop a vaccine against the parasite that is affordable, safe, and effective, in order to control malaria for the long term and possibly eradicate it.

The RTS'S/AS01 vaccine was created for the pre-erythrocytic stage of *Plasmodium falciparum* in order to activate the immune system against the parasite as soon as it enters the human bloodstream, preventing the sporozoites from invading human hepatocytes, eradicating infected hepatocytes, or impeding

Plasmodium development, making it impossible for the parasite to invade red blood cells (Garcon et al., 2007).

The aim of this review to overview the current malaria vaccine (RTS'S/AS01) which was recently approved by WHO.

2. Literature Review

Malaria, sometimes called the "King of Diseases", is caused by protozoan parasites of the genus *Plasmodium*. The most serious and sometimes fatal type of malaria is caused by *P. falciparum*. The other human malaria species, *P. vivax*, *P. ovale*, *P. malaria*, and sometimes *P. Knowles* can cause acute, severe illness but mortality rates are low. Malaria is the most important infectious disease in tropical and subtropical regions, and continues to be a major global health problem, with over 40% of the world's population exposed to varying degrees of malaria risk in some 100 countries (Bell, 2005). The number of malaria cases worldwide seems to be increasing, due to increasing transmission risk in areas where malaria control has declined, the increasing prevalence of drug resistant strains of parasites, and in a relatively few cases, massive increases in international travel and migration (Pasvol, 2005). Clinical diagnosis is based on the patient's signs and symptoms, and on physical findings at examination. The earliest symptoms of malaria are very non-specific and variable, and include fever, headache, weakness, myalgia, chills, dizziness, abdominal pain, diarrhea, nausea, vomiting, anorexia, and pruritus (Tangpukdee et al., 2009).

3. Life Cycle of Malaria Parasite

Malaria is a preventable and treatable, but deadly disease caused by infection of red blood cells by *Plasmodium* parasites, transmitted to humans through the bites of infected female *Anopheles* mosquitoes the stage at which sporozoites pass from the mosquito bite via the blood to the liver (Figure 1). About 50-100 sporozoites are estimated to be injected in the skin during a blood meal by an infected female *Anopheles* mosquito (Graewe et al., 2012). Over a couple of hours, about a third of inoculated sporozoites pass through the dermis, enter the blood stream and reach the liver. The sporozoites travel to the liver and invade liver cells for 5-16 days. Single sporozoites undergo asexual reproduction and produce a thousand merozoites (Yamachi, 2007). The merozoites may re-infect the liver or enter the bloodstream and infect the RBC and become trophozoites (Caraballo, 2014). These trophozoites is at first disc-like, then becomes ring-shaped and at last, becomes amoeboid stage which undergo multiple fission produce merozoites which re-infect RBC. The parasite-infected red blood cell can lead to symptoms such as vomiting, loss of appetite, headache, myalgia, chills, and sweating, as well as respiratory symptoms and this can cause a milder hemolytic anemia. Some young merozoites develop into male and female gametocytes which are ingested by the mosquito and reproduce sexually to form a diploid zygote, that develops into ookinetes burrow in the midgut wall of the mosquito to form oocysts (Skwarczynski, 2020). The oocysts eventually burst, releasing sporozoites into the body cavity

that that travel to the mosquito's saliva glands. The cycle continues when the mosquito bites an appropriate host, injecting the sporozoites into the animal’s bloodstream (Ouattara, and Laurens .2015).

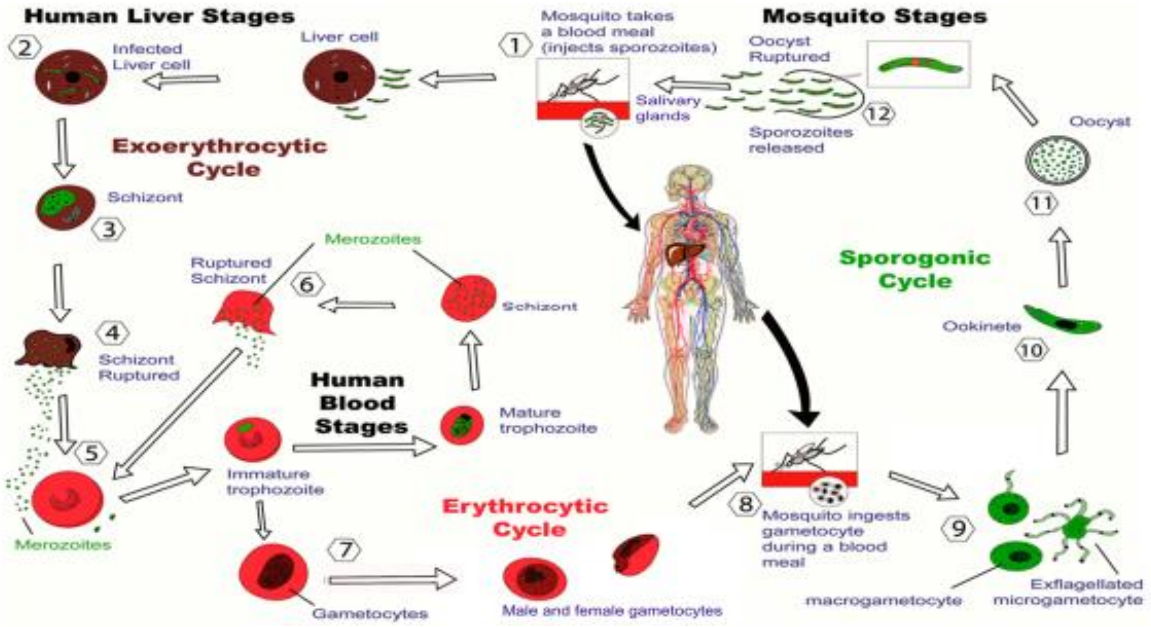

Fig. 1 life cycle of malaria parasite.

4. Type of Vaccines

4.1 Erythrocytic Vaccine

These vaccines act when the merozoites are released from the liver (after completion of the pre-erythrocytic stage) and enter the blood to infect erythrocytes. They are also referred to as blood-stage vaccines. Their goal is to stop the invasion of red cells and prevent the parasite’s asexual reproduction. They produce the antibodies that target the merozoite surface proteins, thereby

halting red cell's invasion. Besides, blood-stage vaccines may also target the merozoite surface proteins, thereby halting red cell's invasion. Besides, blood-stage vaccines may also target the variant surface antigens of the erythrocyte membrane (Sirima et al., 2011).

4.2 Transmission Blocking Vaccines

They prevent the transmission of infection from patients to mosquitoes and halt the spread of the disease. At the end of the erythrocytic stage, some of the merozoites differentiate into sexual stages. These are fed upon by mosquitoes and complete the sexual cycle in the gut. These vaccines incorporate surface antigens of sexual forms of the parasite (gametes and zygote) and generate antibodies that prevent sexual reproduction of the parasite by blocking either the fertilization of the gametes or the development of the zygote into sporozoites (Kubler et al., 2007).

4.3 RTS RTS,S/ASOI (Mosquirix®) Vaccine

RTS RTS,S/ASOI (Mosquirix®) is a malaria vaccine that targets the pre-erythrocytic stage of *P. falciparum*, to activate the immune system against the parasite (Figure 2), as soon as it enters the human bloodstream, preventing the sporozoites from invading human hepatocytes, eliminating infected hepatocytes or impairing the development of *Plasmodium*, making it impossible for the parasite to invade red blood cells (Garçon et al., 2007). RTS,S consists of two proteins, RTS and S, simultaneously expressed in genetically engineered *Saccharomyces cerevisiae* yeast cells (Chackerian, 2007). The vaccine (RTS,S antigen adjuvanted with

AS02A) was developed by a public-private partnership in 2001 between GlaxoSmithKline (GSK) and PATH's Malaria Vaccine Initiative, with support from the Bill and Melinda Gates Foundation to PATH. (The goal of the partnership is to develop RTS,S for infants and young children living in malaria-endemic regions in sub-Saharan Africa (Ema, 2013).

5. History of Malaria Vaccine

In 1967, mice were immunized with *Plasmodium berghei* sporozoites attenuated by X-radiation through studies carried out at the New York University by Ruth Nussenzweig et al.. The results showed that when these animals were challenged with non-irradiated sporozoites, there was a decreased infectivity of sporozoites to infect hepatocytes. Later, the same result was observed in non-human primates (Nussenzweig et al.,) In 1987, new formulations of the malaria vaccine were developed, as well as other adjuvants that were tested to improve their performance. A vaccine containing the recombinant protein from the tetra peptide from the CSP repeat region was developed and tested in mice using aluminum hydroxide (Alum) (Hollingdale et al., 1987). In 1995, one of the first phase I studies in human immunization was carried out using the new formulation of the RTS'S vaccine. The test was carried out on 20 volunteers without malaria, 10 of whom received RTS'S/ Alum and 10 received the RTS'S/Alum/MPL vaccine (Gordon et al., 1995). In 1997, new tests were reinitiated with the RTS'S vaccine, only this time the CSP protein in addition to fused to HBsAg was expressed together with unused HBsAg (Stoute et

al., 1998). From March 2009 to January 2011 a phase 3 study was carried out with infants aged five to 17 months and infants aged six to 12 weeks in eight countries, in Sub-Saharan Africa. In 2019, the most recent phase 3 study with the RTS'S/ AS01 vaccine was carried out with a population of 109 infants aged six to 12 weeks and 86 infants aged 5 to 17 months. Data from this clinical trial found that vaccination with RTS'S/AS01 generated antibodies predominantly of the subclasses IgG1 or IgG3, with lower levels of IgG2 and IgG4 (Dobano et al.,2019).

6. The Structure of Circumsporozoite Protein (CSP)

CSP is a secreted protein of the sporozoite stage of the malaria parasite (*Plasmodium* sp.) and is the antigenic target of RTS,S and other malaria vaccines (Porter et al. 2013). The amino-acid sequence of CSP (Figure 2) consists of an immunodominant central repeat region flanked by conserved motifs at the N- and C-termini that are implicated in protein processing as the parasite travels from the mosquito to the mammalian vector (Aldrich et al., 2012). The amino acid sequence of CSP was determined in 1984 (Dame et al., 1984; Enea et al., 1984).

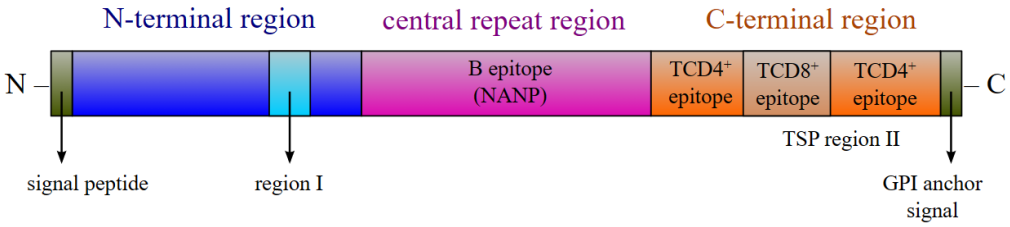

Fig. 2 CPS structure of *P. falsiparum*

The structure and function of CSP is highly conserved across the various strains of malaria that infect humans, non-human primates and rodents. It can first be detected in large quantities as sporozoites are forming within oocysts residing in the midgut walls of infected mosquitoes. Upon egression from mature oocysts, sporozoites begin migrating to the salivary glands, and CSP is known to be an important mediator of this process. Additionally, CSP is involved in hepatocyte binding in the mammalian host. Here, the N-terminus and central repeat region initially facilitate parasite binding (Rathore et al., 2002). On the hepatocyte surface proteolytic cleavage at region 1 of the N-terminus exposes the adhesive domain of the C-terminus, thereby priming the parasites for invasion of the liver (Coppi et al., 2005). CSP is an approximately 58 kD soluble protein (Marques-da-Silva et al., 2020).

7. Structure of Malaria Vaccine

RTS,S, is a recombinant circumsporozoite protein (CSP) that are fused to hepatitis B viral surface protein, such that virus-like (1oyen, 2017). Circumsporozoite protein (PfCSP) covering the surface of the infecting sporozoite (2Rts 2). The CSP (a 412 amino acid protein) is the principal antigen on the sporozoite's surface and is also present early in hepatic infection> *Plasmodium falciparum* CSP as the major component of sporozoite coat 4 CS protein fused to the hepatitis B surface antigen as carrier matrix for the Furthermore, the RTS,S/AS01 candidate induced CS specific

antigen 6. The “R” stands for the central repeat region of the CSP ; the “T” stands for the T-cell epitope of the CS antigen; and first “S” for "Surface" portion which when co-expressed on yeast cell while the next “**S**”. stander for the hepatitis B surface antigen 7. RTS,S is administered with the adjuvant AS01-the leading formulation for induction of high antibody concentrations in humans (Duffy and Partick, 2000). Novel AS01 adjuvant has received a positive opinion from the European Medicines Agency. The Adjuvant Systems eventually selected and used in most of the clinical studies described in the following sections, belong to the AS01 and AS02 families Both include the immune response enhancers (MPL) and QS219 and are formulated either as a liposome-based adjuvant in the case of S01, or an oil-in-water emulsion-based adjuvants for AS02 (Garcon, 2007).

8. Mode of Vaccine Action

RTS.S/AS01 vaccine is formed by tandem repeat region of the CSP, mainly copies of the four amino acid sequence NANP combine with adjuvam-AS01.23. It induces a strong IgG antibody response to the conserved central repeat region of the CSP and potent T-cell (CD4+) response (Ballou and Cahill, 2007) (Figure 3). Antibody levels reach high concentration often hundreds of micrograms per milliliter (White et al., 2015). The levels correlate with protection against infection or clinical malaria in several settings, but the absolute threshold level for protection has not been clearly defined yet. After vaccination, APCs take up RTS,S antigen

and, in the draining lymph node, present processed RTS,S-derived peptides via HLA-II T-cell receptor (TCR) interactions. From these interactions and from CD40-CD40L interactions, CD4 T cells are stimulated to produce Interleukin-2 (IL-2). This IL-2 then activates Natural killer cells (NK) and helps B cells to proliferate and produce antibodies, as well as inducing T-cell proliferation through a positive feedback loop (B). Upon re-encounter with CSP in the draining lymph nodes, (derived from RTS,S or sporozoites), APC present CSP derived peptides to CS-specific CD4+ T cells.

NKcells, in the proximity of IL-2 secreted by CD4 T cells are activated and start secreting Interferon gamma (IFN-y). This IFN-y may signal to the APC to produce IL-12 and IL-18,115-116 which in turn further promotes IFN-y production by NK cells in a positive feedback loop. The IFN-y produced by NK cells may further activate CD4 T cells. Death of infected cells can then be induced by NK cells through released IFN-y or degranulating cytotoxic molecules (C). In the liver, sporozoites traverse from the sinusoidal capillary lined with liver sinusoidal endothelial cells (LSECS) through (a few) Kupffer cells (KC) before infecting a hepatocyte (HC) (dashed line). CSP peptides are presented by Kupffer cells to memory or activated CD4 T cells, which start secreting IL-2. This IL-2 activates liver NK cells, which are further activated by IL-12 secreted by the Kupffer cells. The NK cells then also secrete IFN-y and cytotoxic degranulation molecules (Moris et al., 2018).

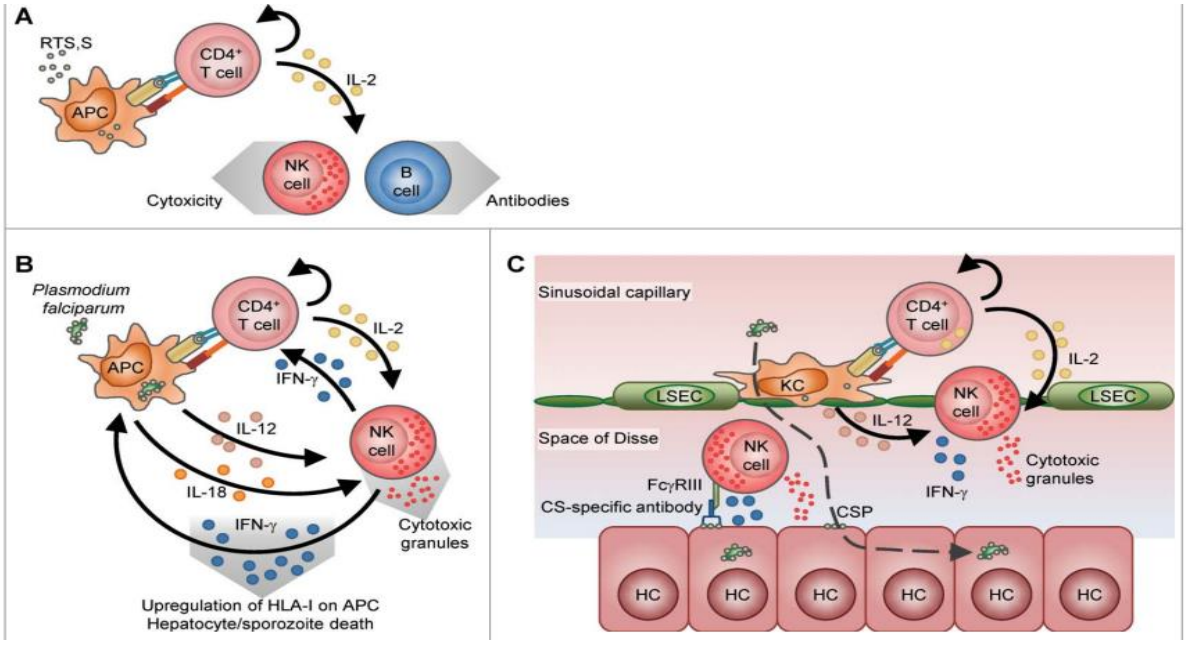

Fig.3 Mode of action

9. The Doses Recommended by WHO

WHO recommends RTS,S/AS01 in a 4-dose schedule for the prevention of *P. falciparum* malaria in children from 5 months of age living in regions with moderate to high malaria transmission, as defend by WHO (2022a & 2022b). The schedule includes three primary doses with a minimum interval of 4 weeks between doses, followed by a booster dose approximately 12–18 months after the third dose. An optional 5-dose strategy may be considered in areas with high seasonal malaria transmission or areas with perennial malaria transmission with seasonal peaks. This strategy includes three primary doses administered at monthly intervals and two annual booster doses administered prior to peak malaria transmission season (WHO, 2022a & 2022b).

10. Efficacy of Malaria Vaccine

With the RTS.S/ASO1 vaccine success in eliciting strong immunogenicity a double-blind, phase IIb, randomized controlled trial was conducted in above two thousand children aged 1-4 years in southern Mozambique in 2003-2004 (Alonso et al., 2004). It found that malaria's incidence rate was 37% lower than in controls during the first six months after the third vaccine dose the vaccine efficacy, Calculated as one minus the incidence rate ratio, was 27% for all clinical episodes and 58% for severe disease. An additional single-blind follow-up for 12 months emonstrated the vaccine efficacy of 29% for all clinical episodes and 39% for severe malaria (Alonso et al., 2005). RTS,S/AS01 vaccine efficacy against the first and only episode of clinical malaria over 12 months after the third dose was 31.1% in infants and 55.8% in children (Chen ,2010). With the encouraging results of early trials, a phase III randomized trial of RTS,S/A501 vaccine (a collaboration between a private foundation, a vaccine manufacturer, and a public health agency) was conducted between 2009 to 2014 in seven sub-Saharan countries (Burkina Faso, Gabon, Chana, Kenya, Malawi, Mozambique and the United Republic of Tanzania) (RTS,S Clinical trials, 2015). The vaccination schedule consists of three baseline doses of 0.5 ml administered intramuscularly at 0, 1, and 2 months, followed by the fourth dose (booster) at 18 months after. The three doses had an efficacy of about 28% in children (age 5-17 months) and about 18% in infants (age 6-12 weeks) against clinical malaria during a

median follow-up after the first dose, 48 months for children and 38 months for infants with a booster dose at 20 months after dose 1, efficacy increased to about 36% in children and about 26% in infants (Bejon et al.,2008). Phase III clinical trials were conducted in Africa in May 2009 and concluded in early 2014. The trial involved 15,459 infants and children in 11 cities in 7 seven African countries (Burkina Faso, Gabon, Ghana, Kenya, Malawi, Mozambique and Tanzania). This clinical trial is the largest malaria vaccine trial ever conducted in Africa (Moncunill et al., 2017). Clinical results in a phase III trial showed a reduction in malaria morbidity in 50% of samples of infants and toddlers aged 5-17 months at the first vaccination. Further analysis was carried out by re-vaccinating RTS after 18 months and it was found that infants and toddlers experiencing clinical malaria cases were reduced by 46% (Campo et al., 2015). These results were achieved by the presence of malaria interventions such as the use of bed nets with insecticides used by 80% of trial participants (Vidal et al., 2018). In the primary series of third dose vaccination, malaria cases were reduced by 26% The fourth dose of RTS,S was administered after 8 months from the primary dose and cases of malaria were reduced by 39% Administration of a fourth dose provides long-term protection against clinical malaria (Campo et al., 2015). Therefore, in 2017 the status of the RTS,S/AS01 vaccine entered phase four, namely the phase to see the effectiveness and side effects of the vaccine if used for a long period of time. This MVIP started in early 2018 (Leitao et al., 2013).

11. The Safety and tolerability profile of RTS,S/AS01

RTS,S/AS01 had acceptable safety and tolerability profiles in phase 3 trials (RTS,S Clinical Trials Partnership 2015; Otieno et al. 2020 and Chandramohan et al., 2021). In a pooled analysis of >11,000 children vaccinated with three doses of RTS,S/AS01, the very common (incidence ≥1/10) adverse reactions occurring within 7 days post-vaccination were fever (incidence 27%), irritability (14%) and injection site reactions, such as pain (16%) and swelling (7%) (GlaxoSmithKline, 2020). This section mainly focuses on data for children aged 5–17 months in the pivotal trial (RTS,S Clinical Trials Partnership, 2015; Guerra Mendoza et al., 2019; Tinto et al., 2019 and Otieno et al., 2020).

12. Conclusion

The quest for a licensed effective vaccine against malaria remains a global priority. Even though classical vaccine design strategies have been successful for some viral and bacterial pathogens, little success has been achieved for *Plasmodium falciparum*, which causes the deadliest form of malaria due to its diversity and ability to evade host immune responses. Nevertheless, recent advances in vaccinology through high throughput discovery of immune correlates of protection, lymphocyte repertoire sequencing and structural design of immunogens, provide a comprehensive approach to identifying and designing a highly efficacious vaccine for malaria WHO recommends RTS,S/AS01 in a 4-dose schedule for the prevention

of *P. falciparum* malaria in children from 5 months of age living in regions with moderate to high malaria transmission, as defined by WHO 55.8% the efficacy of vaccine of infants aged five to seventeen months and 31.3% of infants aged six to twelve weeks had parasite protection that was effective Although its impact has been little thus far, it is anticipated to grow in the future.

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