Status in Malaria Vaccine Development: Basic aspects of Vaccine mechanism of actions, Vaccine pipelines, Stage oriented immune response 'Challenges and Opportunities'.

Review

Solomon Taddese[1] Mullugeta Aemero[2] Eyasu Makonnen[3] Melkam Abaye [4]

[1] medihinsolom@gmail.com, [2] bmeskel@gmail.com, [3] eyasumakonnen@yahoo.com, [4] melk12ab@gmail.com

[1, 2, 4] *Department of Medical parasitology, College of Medicine and Health Sciences, School of Biomedical and Laboratory Sciences University of Gondar, Gondar, Ethiopia*

[3] *Departments of Pharmacological Science, College of Health Sciences, Addis Ababa University, Addis Ababa, Ethiopi*

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ABSTRACT

Malaria is considered as a systemic syndrome caused by infection of the red blood cells by intracellular protozoan parasites of the genus Plasmodium and is transmitted by the bite of infected and physiologically excited female anopheline mosquito, which feeds on mamamalian blood to produce and mature its eggs. Vaccination is believed to be one of the most effective approaches to tackle morbidity and mortality related malaria and its approach targets the malaria life stage factors like the pre-erythrocytic*proteins (RTSs, ChAd63/MVA, METRAP, PSPZ, PfceITos…), the blood stage proteins (EBA175, AMA$_1$, GMZ$_1$, P$_{27A}$, MSP$_3$, MSP$_1$, RH$_5$, sexual stage proteins (Pfs$_{25}$, Pfs$_{48}$, Pfs$_{230}$ and Multi-stage /multi-epitope/antigen combination vaccines ((PfCP-2. 9 chimeric (AMA1 and MSP1–19)). Even if the vaccine trials against malaria was began early in 1930s, currently the one most advanced* pre-erythrocytic*vaccine, RTS, S vaccine has been launched with good safety profile (efficacy of 30-50%). New approaches like combining different types of adjuvants into antigen-specific formulations improved efficacy of a particular vaccine and its formulations offer a wide spectrum of opportunities in malaria vaccine research. Frequent and multiple infections gradually lead to the development of anti-parasite immunity which results in very low or undetectable parasitemia in malaria-infected individuals. Sterilizing immunity against malarial parasite, though never fully achieved, results in a high degree of immune response, low levels of parasitemia, and an asymptomatic carrier status For unsuccessful trials of malaria vaccine, there are so many challenges that are associated to logistic (high cost-effective public health intervention to control and regulate pathway complicity), immunologic (polymorphism of the malaria parasite antigens in each life stage, the immune evasion strategy of the parasite…), and technical challenges related to miss identification of malaria vaccine candidates, selection adjuvants and route of administration. Although there were tackles to malarial vaccine trials, it was reported that there are fine opportunities to proceed on the track.*

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1. Introduction

1.1. Malaria

Malaria is a systemic syndrome caused by infection and lysis of the red blood cells with intracellular protozoan parasites of the genus *Plasmodium*. It is primarily caused by four species of the protozoan parasite *Plasmodium*: *P. falciparum*, *P. vivax*, *and P. malariae* and *P. ovale*, which are transmitted by different species of infected female anopheline mosquitoes, which feed on mammalian blood to mature conceived eggs [1].

Currently, a fifth species, *P. knowlesi,* zoonotic origin, was diagnosed and reported, and it represented a small percentage of infections in human. However, it has not been established whether human-mosquito-human transmission can occur or not [2]. Despite the effort done to manage malaria, reports presented that globally malaria infected 212 million people and caused an estimated deaths of 429 000 people yearly across the world. Africa, a region believed to share a major disease burden, contributes to more than 90% of world malaria cases and death per year [3].

1.2. Vaccination

Vaccination has been one of the most effective alternative approaches of preventing and controlling infectious diseases and it continues to be a lot contributor to qualify immune profile of human body system. The significant progress and success in vaccines development and trials that have been work against disease like polio, measles, diphtheria, tetanus, rabies and the likes, and the complete eradication of smallpox in humans confirmed the potential and the contributions of vaccine in reducing the global health problem of infectious diseases [4]. However, despite the success gained and the effort done for some diseases, there are tackles correlated to logistical and technical issue that remained to be solved to develop efficient malaria vaccines that might potentially provide an important tool for use in malaria elimination and eradication programs [5].

Recent vaccine development trials have led to a new era of vaccine advancement in general and for malaria in particular. Adjuvants formulation and vaccine delivery systems are becoming increasingly more important for the development of a new generation of vaccines. Combining different types of adjuvants into antigen-specific formulations improved efficacy vaccine and its formulations. These new approaches offer a wide spectrum of opportunities in malaria vaccine research with direct applications for the near future. It is well known that purpose of vaccination

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of malaria strategies is to induce protective memory immune responses in advance of infection, and to provide protection in the case of encountering the disease-causing agent again. Malaria vaccine development is an active research area with enormous challenges and opportunities as well [5].

1.3. Malaria parasite life cycle

The malaria parasite exhibits a complex life cycle and stages involving invertebrate vector (anopheline mosquito) and vertebrate host (human). The four types of plasmodium species infect humans: *P. falciparum, P. vivax, P. ovale* and *P. malariae* exhibiting a similar life cycle show only minor variations. Malaria cycle begins pre-erythrocytic stages, a stage that contains injection of the sporozoite by the bite of an infected female anopheline mosquito, and the rapid homing of the sporozoite into the hepatocytes within few minutes to a few hours [6]. So the infection of malaria is initiated when sporozoites are inoculated with the saliva of a feeding mosquito containing different infection facilitating molecules. The injected sporozoites, via the circulatory system mainly and lymphatic system partly, are carried to the liver and start to invade hepatocytes. This intracellular parasite in the liver undergoes an asexual reproduction (exo-erythrocytic schizogony) within the hepatocyte. Exo-erythrocytic schizogony culminates in the production of merozoites that finally are released into the bloodstream in the form of merozoites after brusting out the liver cells. The merozoites then, start to infect the red blood cells in the circulatory system. On the other hand a proportion of the liver-stage parasites from *P. vivax* and *P. ovale* pass through a dormant period called the hypnozoites instead of immediately undergoing liver stage asexual replication. Later these hypnozoites will reactivate several weeks to months (or years) after the primary infection of hepatic cells and are responsible for relapses malaria disease. The merozoites released from the asexual replication in hepatocytes invade erythrocytes (blood stage or erythrocyte stage) [7] and undergo a trophic period in which the parasite enlarges. The early trophozoite in red blood cells are often referred to as 'ring form' because of its morphology become enlarged as they have an active metabolism including the ingestion of host cytoplasm and the proteolysis of hemoglobin into amino acids. There resulted a schizont from multiple rounds of nuclear division without cytokinesis period. When mature merozoites bud from the mature schizont, also called a segmenter, is released following rupture of the infected erythrocyte. Invasion of erythrocytes reinitiates another round of the blood-stage replicative cycle. The blood stage is responsible for the pathology of malaria that expressed in

intermittent fever paroxysms because of the synchronous lysis of the infected erythrocytes. *P. malariae* exhibits a 72 hours periodicity of fever, whereas the other three species exhibit 48 hours cycles. However, *P. falciparum* often exhibits a continuous fever rather than the periodic paroxyms. Relatively *P. falciparum* also is responsible for more morbidity and mortality.

The increased virulence of *P. falciparum* is due in part to the higher levels of parasitemia associated with *P. falciparum* infections and the complications are associated with *P. falciparum* because of the sequestration of the trophozoite and schizont-infected erythrocytes in the deep tissues of micro vessels of vital organs [7].

As an alternative to the asexual replicative cycle, the parasite differentiates into sexual stage known as extracellular macro- or microgametocytes [8] that are relatively larger stage of parasites life cycle and can fill up the erythrocyte cytoplasm, but only contain one nucleus. Ingestion of gametocytes by the mosquito vector induces of gametes into its gut and let an escape from the host erythrocyte. A drop in temperature, an increase in carbon dioxide, mosquito metabolites, stress factors such as lack of nutrition and host immune pressure force merozoites to develop into gametocytes are different contributing factors for the induction of gametogenesis. Microgametes, formed by a process known, as exflagellation are flagellated forms, will fertilize the macrogamete to for a zygote. The zygote develops into a motile ookinete [9, 10], which can penetrate the epithelial cells of the gut of mosquito and develops into an oocyst. The oocyst undergoes multiple rounds of asexual replication resulting in the production of sporozoites. Following rupture of the mature oocyst, the sporozoites are released into the hemocoel (i. e., body cavity filled with fluid) of the mosquito for maturation. Then after maturation, sporozoites migrate to and invade the salivary glands, thus completing the life cycle. Generally, malaria parasites undergo three distinct asexual replicative stages (exo-erythrocyticschizogony, blood stage schizogony, and mosquito stage sporogony) resulting in the production of invasive forms (merozoites and sporozoites). A sexual reproduction occurs with the switch from vertebrate to invertebrate host and leads to the formation of the invasive ookinete. All invasive stages are characterized by the apical organelles typical of apicomplexan species.
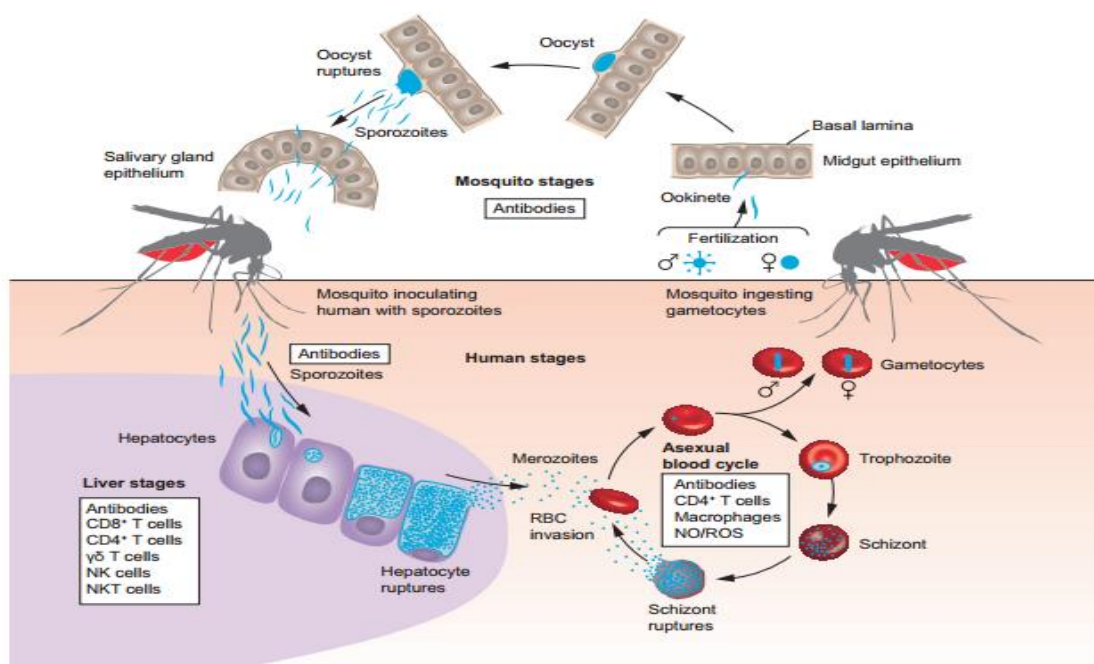
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Figure 1. Life cycle of the malaria parasite. (Source: Arama, C. and Troye-Blomberg, M., 2014).

Gametocytes develop into extracellular gametes in the midgut of the mosquito vector when taken in a blood meal [11] from an infected person to undergo fertilization and continue development in the mosquito. Although most gametocytes remain within the host erythrocyte until they are taken up during a blood meal ingested by a female anopheline mosquito, some of the infected erythrocytes rupture in the host's reticuloendothelial system and present gametocyte-specific antigens to the host's immune system [12]. The aim of this review is to assess the status, challenges and opportunities of malaria vaccines trials of different types and their working mechanism

2. Immune response against malaria infection

Frequent and multiple infections gradually lead to the development of anti-parasite immunity which results in very low or undetectable parasitemia in malaria-infected individuals. Sterilizing immunity developed against malarial parasite, though never fully achieved, results in a high degree of immune response, low levels of parasitemia, and an asymptomatic carrier status [13, 14].

The presence of genetically and antigenically distinct strains of the malaria parasites in a given focal area and the occurrence of clonal antigenic variation during the course of infection force the host to mount immune response against these different strains and antigenic variants. It is

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well understood that the acquired anti malaria immunity has been demonstrated to be strain specific, species specific, stage specific, with cross reactivity, very slow, age associated and not very effective. Immune attack involvement is reported to high in the erythrocytic stage in contrast to *Pre-erythrocytic* stage, and main r immune effectors in the *Pre-erythrocytic* and erythrocytic stages are CD8+ T cells and antibodies respectively [15].
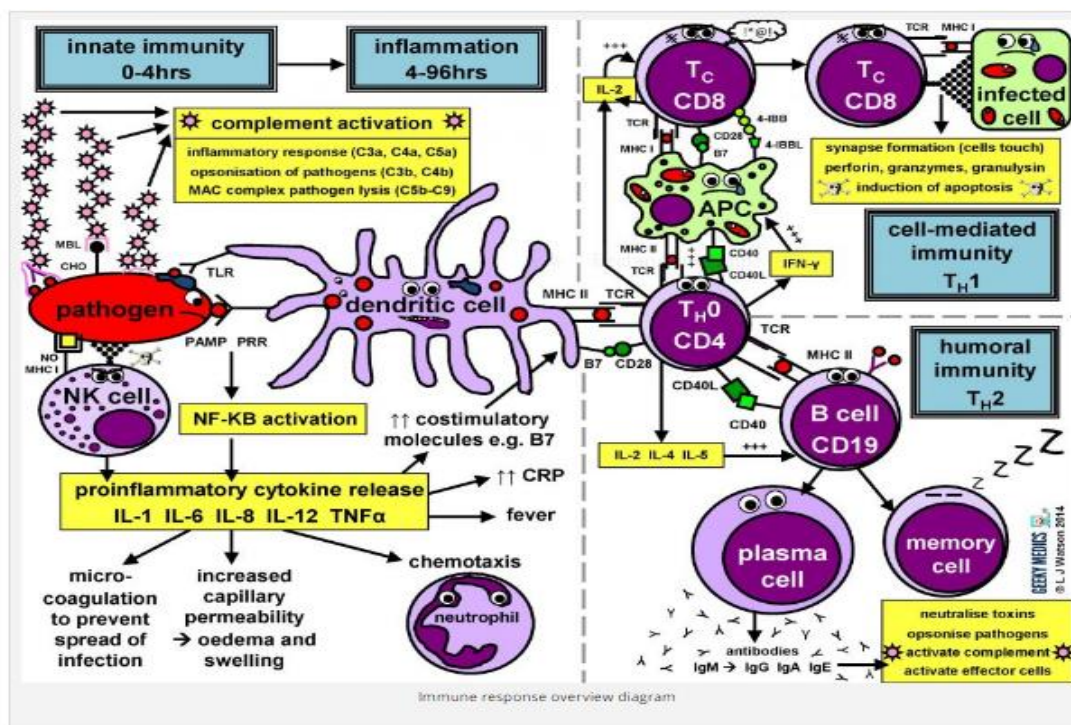

Figure 2- Over view of the immune system to malaria parasite (source –Watson L, 2014)

2.1. Skin as Physical Barrier

The skin maintains physical barrier and is considered the first line of defense against malaria parasites and other pathogens. After inoculation, sporozoites stay in the skin for several hours because of defence from skin immune system. Antibodies and the dendritic (Langerhans cells) found in the skin tissues inhibit sporozoite motility across the dermis [16]. Because of this immune response, approximately 50% of the sporozoites will get damage at the inoculation site of the skin [17]. The sporozoite proteins (SPECT1 and SPECT2) were reported to be necessary to pass the skin barrier, cell traversal, and migration to the liver [18]. However, few sporozoites evade destruction by phagocytes in the skin and growth is arrested in non-phagocytic cells in the host dermis [19].

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2.2. Immune Response to Pre-erythrocytic-Stage Parasites.

Immune response at the Pre-erythrocytic stage, also called liver stage, is targeted on free sporozoites and infected hepatocytes. Antibodies response against free sporozoites and circum sporozoite protein (CSP) are important to prevent invasion of hepatocytes by neutralizing proteins required for cell invasion. An antibody also activates complement fixation, phagocytosis, and lysis by cytotoxic NK and NKT cells. It also recognizes parasite neoantigens at the surface of infected hepatocytes and kills through an antibody-dependent cell-mediated mechanism by Kupffer cells and NK cells [20]. CD8+ T cells producing interferon-γ are mainly involved in killing of intrahepatic parasites. Other cells like NK, NKT, and γδT cells also kill intrahepatic parasites through secretion of type I interferons and IFN. Specially, NK cells in the liver (more frequent than in any other tissues) possess diverse PRRs; which do not only recognize PAMPs, but also damage-associated molecular patterns. NK cells express ligand-responsive TLRs 1–4 and TLRs 6–9 and upon activation, they opsonize by antibodies, release perforin and various granzyme from their intracellular vesicle stores by exocytosis. In addition, activated NK cells secrete numerous cytokines, as, e.g., IFNγ, and are therefore considered as to shape subsequent immune responses including modulation of MHC. Kupffer cells (KCs), liver-resident macrophages are located in the sinusoid lumen, adhere to LSECs and represent about 30% of the non-parenchymal liver cells, express different surface receptors allowing the detection, binding, and phagocytosis of malaria parasite. For instance, KCs express ligand-responsive TLRs 2–4 and TLR9. Even if KCs are involved in waste disposal, which also includes phagocytosis of activated and apoptotic "self" cells, as, for example, neutrophils, platelets, and T cells after immune fighting. Upon activation, KCs produce a series of cytokines that include TNFα, IL-1β, IL-6, IL-12, and IL-18 against malaria parasite. Moreover, KCs are capable of antigen presentation. They express MHC I, MHC II and co-stimulatory molecules required for activation of T cells. In particular, KCs can activate natural killer T (NKT) cells patrolling the sinusoids γ. Dendritic cells (DCs) are concentrated around the portal triad and around the central veins of the liver and have ability to activate T cells and may be important for induction and maintenance of immune tolerance T cells. The major function ascribed to hepatic DCs is to control the balance and portal autoimmune diseases and phagocytosis of the parasite. DCs are also identify pathogens by recognizing pathogen-associated molecular patterns (PAMPs) using pathogen recognition receptors (PRRs). Toll-like receptors (TLRs) are an

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example of a PRR. And once they have identified malaria organisms, they internalize, kill and digest them down into their component proteins then present the digested protein antigens to the cells of the adaptive immune system via major histocompatibility complexes (MHCs) on their surfaces [13, 21, 22].

 On the other hand unlike viruses and bacteria, *P. falciparum* malaria parasites can trigger type I IFNs in the absence of Toll-like receptors (TLR3 and TLR4) and their signaling proteins (MyD88 and TRIF); rather, they use melanoma differentiation associated gene 5 protein (MDA5) which activates the transcription factors IRF3 and IRF7 [23]. Very recently, an exo-erythrocytic form (EEF) RNA was also reported to be recognized by MDA5 in hepatocytes, triggered a type I IFN response in the innate immune cells. Other soluble substances produced by HCs are complement components, e.g., C3, that opsonizes pathogens for elimination, and C9, that forms the membrane attack complex for final microbe lysis. Moreover, HCs produce a number of circulating growth factors and inflammatory cytokines, amongst which is IL-6 ("classic signaling" is restricted only to HCs and immune cells) that in turn induces massive production of APPs by HCs [23].

Host iron regulatory hormone hepcidin, which impairs the growth of sporozoites, is also produced by unknown mechanisms of the human host [19]. Killing of infected hepatocytes and blocking of invasion by CD8+ T cells and antibodies, respectively, are bottleneck phases that could be an alternate targeted by vaccine [24].

2.3. Immune Response to Erythrocytic Stage of Infection.

Adaptive immunity against erythrocytic-stage *P. falciparum* is more complex than the other stages [25]. The release of merozoites from hepatocytes to invade RBCs is responsible for initiation of the erythrocytic stage. At this stage, the immune targets are free merozoites and intraerythrocytic parasites (schizonts). Humoral (antibodies) and T cell responses are important to control merozoites and intraerythrocytic parasites, respectively. Antibodies can opsonise merozoites for uptake by phagocytes or to inhibit invasion of RBCs. In addition to these, Antibody mediates cellular killing, blocks adhesion of infected RBCs to endothelium, and neutralizes parasite toxins to prevent the induction of excessive inflammation. It also marks merozoites for lysis by complement. This stage is also known by proinflammatory cytokine response that activates macrophages [13]. The role of CD8+ T cells in the erythrocytic stage is negligible [25].

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CD4+ T helper cells are also important to produce proinflammatory cytokines that activate macrophages, B cell clones and others like NK cells and γδT cells. CD4+ T helper cells are also involve in direct immune response [18]. Following activation, IFN-γ, perforin, and granzyme produced by NK cells are responsible to kill *P. falciparum* infected RBCs [26].

2.4. Immune Response against Gametocyte.

Antibodies kill gametocyte through complement-mediated lysis and prevent sequestration and maturation of gametocytes in human host body. Antibodies derived from insect host during blood meal are also highly responsible for complement-mediated killing of gametocytes and prevent gamete fusion in mosquito. Nitric oxide produced by macrophages is also important to kill gametocytes [25].

2.5. Anopheline Mosquito's Immune Response.

 Plasmodium falciparum malaria parasite is challenged by several factors before establishing infection in its anopheline mosquito vector. Physical barriers like peritrophic membrane (PM) of the midgut, cuticle of the exoskeleton, and mucosal lining of the tracheal respiratory system are among the major limiting conditions [27]. Capsule formation around the parasite by mosquito melanin also has a protective role [28]. The micro biota found in anopheline mosquitoes, such as Asaia, Enterobacter, Pseudomonas, stimulate a basal innate immune activity against *P. falciparum* infection [29]. Report showed an increased susceptibility to Plasmodium malaria infection in microbe-free mosquitoes. Complement-like or thioester-containing protein (TEP) 1 that circulates in the anopheline mosquito hemolymph is the major arm of defense in the humoral immune response of anopheline mosquito [29]. It forms leucine-rich repeat protein 1 (LRIM1)/anopheline

plasmodium responsive leucine rich repeat protein (APL) 1/TEP1cut complex and gets accumula ted on the ookinete surface for killing. Antibodies also avert ookinete motility, penetration of the midgut wall, and formation of oocyst [26]. The primary immune cells involved in mosquito innate immune response are hemolytic [30]. Hemocytes include granulocytes, oenocytoids, and prohemocyte subtypes that are involved in phagocytosis, melanization, and hematopoietic progenitors, respectively [29]. Other immune effectors released by hemocytes and fat body into hemolymph are also involved in phagocytosis, secretion of antimicrobial peptides, nodule formation, agglutination, encapsulation, and melanization [27]. Reactive oxygen species (ROS) produced by hemocyte is also involved in mosquito immunity against *P. falciparum* [31].

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3. Malaria vaccines trial status

3.1. History of malaria vaccine

Malaria vaccine research began early in the 1930s using inactivated or killed parasites that finally failed to generate a protective immune response. On the next trial with addition of adjuvants demonstrated immunogenicity of malaria vaccine candidates in animal models demonstrated

partprotection.Subsequent vaccine development efforts using rodent malaria models and antigens of irradiated. *P. falciparum* sporozoites to vaccines by mosquito bite have led to the first human malaria vaccine trial with demonstrated efficacy [32, 33].

Later the effort towards generating immunity became impractical for mass vaccination campaigns, and synthetic peptide vaccines based on immunogenic parasite proteins began to be developed in the 1980s. As there is no absolute immunological protection for malaria, continued efforts in vaccine development were time consuming. A series of steps were considered and tried before field-testing in the target population of children in malaria-endemic areas to determine vaccine efficacy. Some of these series of steps are development of a candidate vaccine in the laboratory, testing for safety in animal models, and age de-escalation phase 1 testing in adults and then in children for safety and reactogenicity. These multiple steps took long process and need significant funding support and carry the risk of a negative result. To abrogate this risk, controlled human malaria infection (CHMI), where parasites are inoculated with sporozoites via the bite of infected female Anopheline mosquitoes in well-controlled settings, was used to obtain data on vaccine and drug efficacy in order to support further clinical testing in malaria-endemic areas [35]. Early testing of the RTS, S vaccine using CHMI predicted efficacy in field studies and became important to refine the choice of adjuvant [36]. In the mid- 1970s, the first malaria immunization trials to use experimental challenge by inoculum of parasite to human done by infected mosquitoes were conducted [34, 37].

Next to measure efficacy against clinical malaria in field trials were conducted in the 1990s with the SPf66 vaccine, conjugate vaccine of sequences from *P. falciparum* blood-stage antigens and the circumsporozoite protein (CSP). The trials showed a modest reduction of *P. falciparum* parasitemia in South America where as no protection in Africa [38. 39]. The other effort that includes parasite culture cultivation methods and sequencing of the *P. falciparum* genome have become with hope for the development of a malaria vaccine [39]. However, after more than 35

years of laboratory research and field trials, the only vaccine that has progressed to phase three trials is the RTS, S vaccine, which showed efficacy of 30% in newborns and 50% in children aged 5–17 months [39, 40].

3.2. Malaria vaccine

3.2.1. Liver stage vaccines

Pre-erythrocytic or liver stage vaccines target the clinically silent sporozoite and liver stages of Plasmodium virulent proteins. These vaccines aim to eliminate parasites early infection and, if highly efficacious to induce sterilizing immunity, will avert malarial disease and interrupt transmission. In the 1970s, it was noted that whole sporozoite vaccines were shown to protect humans from *Plasmodium falciparum*, whole organism vaccine approaches that irradiated parasites, genetically modified parasites, and infection in conjunction with chemoprophylaxis (called chemoprophylaxis vaccination or CVac). There is recent progress in manufacturing whole organism vaccines. Results from trials of the PfSPZ vaccine, manufactured by Sanaria Inc. PfSPZ vaccine induces a breadth of responses that includes CD4, CD8, and gamma delta T cells, as well as antibody responses. PfSPZ vaccine also induces antigenic breadth. PfSPZ vaccine efficacy in malaria-naïve adults depends on direct venous inoculation to induce tissue resident T cells in the liver, and the dose determines the degree and durability of homologous and heterologous protection [40–45]. Trials of PfSPZ vaccine confirm efficacy against naturally transmitted parasites. PfSPZ vaccine, currently being tested in 5–12-month-old infants for safety and efficacy against malaria infection, seek to optimize the dose/regimen, incorporate additional vaccine strains, and compare efficacies versus chemoprophylaxis vaccination and genetically attenuated parasite (GAP) vaccines. Report on the development of GAP vaccines involved the deletion of the pre-erythrocytic stage-expressed genes SAP1, P52, and P36 that arrests parasite growth in hepatocytes at the early liver stage. Vaccination using triple gene knockout *P. yoelii* parasites conferred sterilizing immunity to mice [46]. Furthermore, sera from human subjects immunized with the *P. falciparum* triple gene knockout GAP vaccine [47] when passively transferred into humanized-liver mice, protected against infectious *P. falciparum* sporozoite challenge, indicating that antibodies contribute to GAP vaccine immunity. This *P. falciparum* early liver stage-arresting, triple gene knockout GAP vaccine has been shown to be safe in human subjects. When delivered by mosquito bite, during first generation GAP vaccine passes through clinical development, it is thought that the optimal GAP vaccine might allow parasite

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development through late liver stage schizogony with subsequent developmental arrest before formation of infectious exo-erythrocytic merozoites. This GAP vaccine candidate has been shown to induce superior protection against sporozoite challenge as well as stage-transcending protection against a rodent malaria blood stage challenge in mice, both mediated by antibodies and T cells [48].

P. falciparum in late liver stage-arresting GAP vaccine has yet to be generated and is considered a central focus of GAP vaccine research for some researchers. On the other hand, report progress with chemoprophylaxis vaccination (CVac) using sporozoites inoculated by mosquitoes induced both poly functional T cells and antibodies, and conferred long-lasting protection against controlled human malaria infection (CHMI) with homologous parasites. Recently, CVac using cryovialed sporozoites also induced sterilizing immunity in malaria-naïve adults against homologous CHMI, even at accelerated vaccine schedules completed within a month [49]. Future studies will explore CVac strategies that may confer long-lasting heterologous protection by incorporating different *P. falciparum* strains, which will be evaluated for their ability to confer protective efficacy in the field. On the other hand it was described the arc of development for the subunit malaria vaccine RTS, S that completed phase III trials in recent years. The RTS, S vaccine is composed of the repeat region of the circumsporozoite protein fused to the *Hepatitis B virus* surface antigen, and is a djuvanted with the proprietary AS01 adjuvant. Summarized study report showed in its clinical the safety and efficacy in adults, children, and young infants in sub-Saharan Africa [50, 51] in the phase III multicenter efficacy trial that enrolled over 15, 000 children in [47] centers across seven African countries, RTS, S/AS01 vaccination, is estimated to have prevented 829 clinical malaria episodes per 1000 children over 18 months of study follow-up [52]. Future studies will evaluate RTS, S integration into the EPI (expanded program of immunization) schedule. Generally targeting the pre-erythrocyte includes circumsporozoite protein (CSP) present intra vascular, the liver stage antigens (LSA-1) and (LSA-3) which are intra hepatic and sporozoite surface proteins-2/Thrombospondin-related anonymous protein is (TRAP) and CSP and TRAP-related proteins (CTRP) [50].

Table 1. Vaccine targets of malaria at pre-erythrocytic stage

Stage	Antigen	Antigen Description	Vaccine acting	Trial stage

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			Mechanism	
pre-erythrocytic stage vaccines	**RTS, S**	Circumsporozoite protein fused to hepatitis B surface antigen	Inhibit sporozoite motility; prevent hepatocyte invasion	Phase 3 clinical testing
	ChAd63/MVA, METRAP	Chimpanzee adenovirus 63/modified vaccine Ankara, multiple epitope string with thrombospondin-related adhesion protein	Inhibit sporozoite motility; prevent hepatocyte invasion	Phase 2 clinical testing
	PfSPZ	Radiation-attenuated whole organism *P. falciparum* sporozoites	Inhibit sporozoite motility; prevent hepatocyte invasion	Phase 1 clinical testing
	PfCelTOS	*P. falciparum* cell-traversal protein for ookinetes and sporozoites	Inhibit sporozoite motility; prevent hepatocyte invasion	Phase 1 clinical testing
	Recombinant CSP	Recombinant circumsporozoite protein	Inhibit sporozoite mobility; prevent hepatocyte invasion	Preclinical testing
	Genetically attenuated sporozoites	Genetically attenuated whole organism *P. falciparum* sporozoites	Inhibit sporozoite motility; prevent hepatocyte invasion	Preclinical testing

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3.2.2. Blood stage vaccines

Erythrocyte invasion is rapid process and involves a number of parasite protein located on cell membrane of parasite and red blood cells. Most report showed that obstacles to blood stage vaccine development includes antigenic of surface proteins polymorphism of both merozoite as well as infected erythrocyte, redundancy in the merozoites invasion pathways [52] and difficulties expressing conformationally correct sequence of proteins. Vaccine blood stage pre C. G. vents, merozoite multiplication or the invasion of red blood cells and another approach would be to attempt to block the process of erythrocyte adherence to blood vessel walls. This approach is complicated by the lack of MHC molecule expression on the surface of erythrocytes. Blood stage vaccine targets MSP-1, AMA-1 and MP- antigens of merozoite surface proteins and var2 CSA and Var1 CSA members of Pf EMP1 family. Studies so far associate to those antigens suggest that high antibody concentrations are needed to control merozoites invasion in vivo, and existing assays have failed to predict protection in humans in the blood stage vaccine. Vaccine trials on merozoites proteins have been disappointing, but ongoing efforts seek to improve the immunogenicity and functional activity of the antigens. New antigens as well as new antigen combinations might give additive or synergistic activity, and hence are a research priority for several groups. Recent findings from studies of chemically attenuated whole blood stage parasite vaccines indicated the chemical attenuation procedure developed entails incubation of blood stage parasites with a DNA-binding drug (Gentamycin) [53, 54] and thought to affect parasite replication by irreversibly alkylating parasite DNA in poly A rich regions [53]. Immunization of mice with chemically attenuated blood-stage parasites, either as a single dose of *P. chabaudi* or three doses of *P. yoelii*, has induced homologous and heterologous immunity in a CD4+T cell dependent fashion [53, 54, 56]. These vaccines activate peripheral blood CD8+ T cells; but do not confer cross-stage protection, and blood-stage protection remained intact after CD8+ T cell depletion [55]. Similar findings in monkeys [57] prompted a pilot study in humans to assess safety and immunogenicity of *P. falciparum* blood-stage parasites chemically attenuated in vitro. Lysed infected erythrocytes do not induce protective immunity and the requirement for intact infected erythrocytes as an immunogen poses some challenges, such as the risk of induction of antierythrocyte antibodies and ethical issues regarding red blood cell products for some individuals. Novel approaches are under investigation in order to develop semi-synthetic blood-stage vaccines as alternative immunogens that can move forward into human studies [56]. It was

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described progress using the antigen *P. falciparum* RH5 to block merozoite invasion of erythrocytes and prevent blood-stage malaria infection. PfRH5 has been identified as the first highly conserved target from the merozoite to be susceptible to vaccine-induced broadly neutralizing antibody [57]. In monkeys, PfRH5-based vaccines induced antibodies and conferred protection against a virulent heterologous falciparum challenge. Protection was associated with anti-PfRH5 antibody concentration and within vitro parasite neutralizing activity. More than 60% in vitro growth inhibition activity (GIA) was observed with the purified IgG and the minimal serum concentration of anti-PfRH5 IgG required to protect an animal was defined as 200µg/ml. A first generation PfRH5based vaccine is being tested in clinical trials GAP vaccines Whole organism genetic attenuation [57].

Table 2. Vaccine targets blood stage malaria parasite

Target	Antigen	Antigen Description	Vaccine acting Mechanism	Trial stage
Blood stage vaccines	EBA 175	Erythrocyte-binding antigen 175	Target merozoite ligand that mediates erythrocyteinvasion	Phase 1 clinical testing
	AMA1	Apical membrane antigen 1	Target the merozoite's invasion apparatus to prevent erythrocyte infection	Phase 2 clinical testing
	GMZ2	Recombinant Lacto coccuslactis hybrid glutamate-rich protein and merozoite surface protein 3	Target merozoite surface to inhibit erythrocyte invasion	Phase 2 clinical testing
	P27A	*P. falciparum* malaria protein PFF0165c	Target merozoites surface to inhibit erythrocyte invasion	Phase 1 clinical testing
	MSP3	Malaria surface protein 3	Target merozoite surface to inhibit erythrocyte invasion	Phase 2 clinical testing
	SE36	*P. falciparum* serine repeat antigen 5	Target merozoite surface to inhibit erythrocyte invasion	Phase 1 clinical testing
	PfPEBS	*P. falciparum* pre-erythrocytic and blood stage	Inhibit sporozoite motility; prevent hepatocyte invasion; target merozoite surface to inhibit erythrocyte invasion	Phase 1 clinical testing
	MSP1	Malaria surface protein 1	Target merozoite surface to inhibit erythrocyte invasion	Preclinical testing
	Rh5	Reticulocyte-binding protein homologue 5	Target merozoite ligand that help invasion	Preclinical testing

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3.2.3. Transmission blocking vaccines (Tbvs)

Transmission blocking vaccines (TBV) are targeted on erythrocytic sexual parasitic stage antigens that can be categorized into two target antigens groups; as pre-fertilization antigens (Pfs48/45 and Pfs 230 antigens found at the surface of the male and female gametes of malaria parasites) and the other post-fertilization antigens (Pfs 25 and Pfs 28). The benefit regarding to the post-fertilization target antigens is the strong immunogenicity and the limited pre-existing antigenic polymorphism [58]. Fertilization target antigens are surface proteins expressed on the zygotes and mature ookinetes of the malaria parasites representing the second class of malaria transmission [59]. Antibodies directed against Pfs25 completely block pathogen transmission cycle [60] and actually expressed on the surface of late ookinetes, but Pfs28 is protein involved in adherence to the mosquito's gut epithelium [61]. Clinical trials presented the two vaccine candidates Pfs25 (postfertilization antigen) and Pfs230 (pre fertilization antigen) showed intrinsically poor immunogenicity. So scientists tried to conjugate both Pfs25 and Pfs230 to the immunogenic carrier protein Exo Protein (EPA) and administered them with adjuvants formulated with hydrogel established to make the antigen safe and immunogenic in humans. These trials could be able induce functional antibodies that possibly block parasite transmission to mosquitoes in laboratory assays. Comparing and combining Pfs25 and Pfs230 vaccine is now on trials. In studies from Mali, malaria exposure is associated with atypical memory B cells that have decreased effector functions [62]. Th1-polarized T follicular helper cells that exhibit impaired B cell help [63] and moncoytes that have a blunted inflammatory response [65]. It is well known, Tregs (Foxp3+ CD4+ T cell) expand in humans during malaria infection, and Tregs can potentially prevent parasite clearance by inducing a transient hiatus in the parasite-specific CD4 T cell responses. In mice model, the effector CD4 T cell response to *P. yoelii* infection is biphasic and the hiatus coincided with Tregs that up regulated CTLA4. Blockade of CTLA-4 during the T cell hiatus leads to memory responses that confer species-transcending immunity to challenge [64]. Notably, CTLA4 blockade before or after the CD4 T cell hiatus does not improve the CD4 T cell response or control of parasitemia. Generally, Tregs impede acute and long-term immunity against blood-stage malaria through CTLA4 expression, and this Treg activity manifests in a limited time window during the infection. Research on genotype-specific vaccine efficacy, initially noting that many *P. falciparum* antigens have extremely high diversity, including the major sporozoite surface antigen CSP (circumsporozoite protein) targeted by the

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RTS, S vaccine. Using Illumina sequencing data of parasite DNA from the RTS, S trials, it was tested whether the vaccine allele (3D7)-specific "sieve-effect" would result in fewer vaccine-match infections in vaccinated participants. The analysis indicated a greater cumulative efficacy against vaccine-match parasites, and that 3D7 haplotype frequencies varied by study site throughout Africa [65]. The results emphasize that parasite variation can limit the efficacy of malaria vaccines, and highlight the potential for parasite variants that evade vaccines to spread [66, 67] and assessing vaccine activity via standard membrane feeding assays (SMFAs) as well as by feeding mosquitoes directly on vaccines (i. e., Direct Skin Feeding or DSF Assay). Pfs230 vaccine activity is known to be complement-dependent, and therefore fresh complement is required in the SMFA. In an ongoing trial, both Pfs25 and Pfs230 conjugate vaccines are being administered with AS01 adjuvant from GSK based on preclinical studies that this formulation might significantly increase antibody titer and therefore serum functional activity following vaccination. In recent studies Pfs47 appears to function as a "lock and key" to define compatibility between *P. falciparum* parasites and mosquitoes collected from different continents [68] Pfs47 proteins with compatible sequences allow the parasite to evade the Anopheline gambiae immune system, whereas parasites with incompatible sequences achieve only low infection rates. Pfs47 could have an important role in parasite transmission, and therefore might be used as a target for vaccine as antibodies to a specific domain induce strong transmission-blocking activity. Ongoing studies are investigating naturally acquired antiPfs47 antibodies in Malians, and the possibility that the Pvs47 orthologue could be modulating immune evasion in a *P. vivax* model [69].

3.2.4. Multi-stage multi-epitope/antigen combination vaccines

 Most of the malaria candidate vaccines use technologies input like recombinant proteins, synthetic peptides, viral vectors, bacterial vectors, plasmid DNA and attenuated organisms. The vaccines recently under clinical trial are based on the using of single antigen from specific stage of malaria parasite life cycle. To have a better control on the spread of malaria, it will be essential to create a multi-stage specific complex vaccine or several individual stage-specific subunit vaccines singly or in combination using most non-polymorphic region (s) of the promising candidate antigens [70].

One of the vaccines recently developed based on this idea is PfCP-2. 9. This vaccine candidate consists of the C-terminal regions of two leading malaria vaccine candidates – domain III of

apical membrane antigen-1 (AMA-1) and 19-kDa C-terminal fragment of the MSP-1. Both of these regions are least polymorphic portions of AMA-1 and MSP-1, respectively; therefore most conservative antigens for combination vaccine candidate. The two candidates (AMA-1 and MSP-1) enhance the product yield and immunogenicity of the individual components when combined. The protective immune responses induced by either AMA-1 or MSP1–19 are dependent on protein conformation [70]. It is true that, when two antigens are expressed in a combination vector there will be antigenic competition. This vaccine candidate, fortunately, induces antibodies that inhibit blood-stage parasite growth in vitro and results showed no evidence of antigen competition. However, it revealed that sera from animals immunized with combination antigens recognized both the blood-stage parasite and the sporozoite. Pf CP-2. 9 chimeric (AMA1 and MSP1–19) protein previously appeared to be highly immunogenic in rabbits and monkeys. As the anti-AMA1 (III) antibody titre induced by PfCP-2. 9 was more than 15-fold higher than that induced by AMA-1 (III) and the antiMSP1–19 antibody titre was 11-fold higher than that induced by MSP1–19 [70, 71]. It was suggested that PfCP-2. 9 in combination with these recombinant pre-erythrocytic antigens induced antibodies that inhibited growth of blood-stage malarial parasites [72].

Increasinghepotential immunity generated against Plasmodia can be achieved by attempting totar get multiplephasesin the life cycle. This is additionally beneficial in reducing the possibility of resistant parasites developing. The use of multiple-parasite antigens can therefore have a synergistic or additive effect. One of the most successful vaccine candidates currently in clinical trials consists of recombinant antigenic proteins to the circumsporozoite protein [73].

Table 3. Vaccine targets of sexual stage of malaria

Agent	Antigen	Antigen Description	Vaccine Mechanism	Stage
P.falciparum Transmission-blocking vaccines	Pfs25	*P. falciparum* surface protein 25	Inhibit ookinete develop ment in the mosquito midgut	Phase-1 clinical testing
	Pfs48	*P. falciparum* surface protein 48		Pre-clinical testing
	Pfs45	*P. falciparum* surface protein 45		Pre-clinical testing
	Pfs230	*P. falciparum* surface protein 230		Pre- clinical testing

3.2.5. Pregnancy malaria

By inhibiting the expression of parasite surface protein parasite-derived from erythrocyte membrane protein (PfEMP1) from interacting with various vascular endothelial cell–surface

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receptors in human host, there is a possiblity to block the sequestration of parasite-infected erythrocytes and prevent the serious complications such as cerebral malaria or placental malaria [74, 75].

A single PfEMP1 variant, termed VAR2CSA, which is structurally distinct from all other PfEMP1 family members, has been identified to play a key role in sequestration to the placenta by binding to chondroitin sulfate (a component of human connective tissues found in cartilage). The increased ability of multigravidae women to control pregnancy-associated malaria has been attributed to the acquisition of anti-PfEMP1 immunity during successive pregnancies [74, 75]. Trial is on to develop a candidate vaccine based on PfEMP1 antigens aimed at the prevention of pregnancy-associated malaria [75]

Table 4. Vaccine targets of malaria in pregnant woman and *P. vivax* antigens for vaccines

Target	Antigen	Antigen Description	Vaccine Mechanism	Most Advanced Status
P.falciparum Pregnancy associated malaria	var2 CSA	var2 Circumsporozoite Antigenges	var2 CSA inhibition	Preclinical testing
P.vivax Pre-erythrocytic factor	CSP	Circumsporozoite protein	Inhibit sporozoite motility; prevent hepatocyte invasion	Preclinical testing
P. vivax erythrocytic factor	PvDBP	*P. vivax* duffy-binding protein	Inhibit parasite ligand that binds to placental matrix	Phase 1 clinical testing

4. Basic aspects of Vaccinology

One of the basic limitations for the success of malaria vaccines is the problem associated in maintaining durable protection against the parasite following immunization, which in part has been ascribed to poorly immunogenic antigens. Efforts to increase the degree and durability of vaccine protection have included novel adjuvants and effort to improve altered vaccine schedules, dosages and methods of delivery [76. 77, 78]. Even if, long-lived protection

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has not been secured, researchers are becoming interested in looking for the factors that impair the host response to malaria as well as malaria vaccine efficacy. Report of Immuno genentics, emphasized that the immune response to malaria is complex. Clinical immunity only develops after repeated infections with differing parasite strains and may be expressed later in life. Many infected residents of malaria-endemic areas show no sign and symptoms. It was also discussed that it is important to understand the immune responses induced by vaccines to aid future vaccine design and refinement. For example, combining data from phase 2 or phase 3 clinical trials with knowledge of the mechanism of action of vaccine-induced antibodies allows determination of minimum effective antibody concentrations (MEC). Assessing the durability of these strain specific antibodies; specifically, the time that concentrations remain above the MEC could speed up the evaluation of new vaccine formulations. The MEC; for anti-circumsporozoite antibodies induced by RTS, S is very high (in 1mg/ml) and could be maintained for only a few months after vaccination [76] and MECs for other invasion-blocking antibodies (e. g., anti-PfRH5 and anti-PfAMA-1) are of a similar magnitude. These data highlight the need to understand how to induce long-lived, high titer antibody responses that are needed to confer protection. It was also noted that factors that is assumed to be beyond parasite antigenic variation and allelic diversity that may limit vaccine efficacy in endemic areas. Particularly, Plasmodium infections may subvert vaccine-induced immunity. In studies report from Mali, malaria exposure is associated with atypical memory B cells that have decreased effector functions [79] expression of Th1-polarized T follicular helper cells that exhibit impaired B cell help [80] and moncoytes that have a blunted inflammatory response [81]. The studies support additional research to understand the impact of Plasmodium exposure on the quality of vaccine-induced responses, and to explore targeted interventions that could mitigate malaria-driven immunomodulation during vaccination. Mechanisms underlying ineffective control of blood-stage Plasmodium infection, using animal models, showed the importance of CD4 T cell responses in inducing protection against Plasmodium. Blood-stage infection of *P. yoelii* in mice induces exhaustion of the CD4 T cell response. Consistent with this, in humans, *P. falciparum* infection induces higher expression of the inhibitory receptor associated with T cell dysfunction [82] Previous studies have not established a consensus on the role of T regulatory cells (Treg) in the host response to Plasmodium infection, possibly due to variations in the parasite species. However, Tregs (Foxp3+ CD4+ T cell) expand in humans during malaria infection, and Tregs can potentially

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prevent parasite clearance by inducing a transient hiatus in the parasite-specific CD4 T cell responses. In mice, the effector CD4 T cell response to *P. yoelii* infection is biphasic and the hiatus coincided with Tregs that up-regulated CTLA4. Blockade of CTLA-4 during the T cell hiatus leads to memory responses that confer species-transcending immunity to challenge [83] Notably, CTLA4 blockade before or after the CD4 T cell hiatus does not improve the CD4 T cell response or control of parasitemia. Generally, Tregs impede acute and long-term immunity against blood-stage malaria through CTLA4 expression, and this Treg activity manifests in a limited time window during the infection. Professionals spoke on genotype-specific vaccine efficacy, initially noting that many *P. falciparum* antigens have extremely high diversity, incuding the major sporozoite surface antigen CSP (circumsporozoite protein) targeted by the RTS, S vaccine. Using Illumina sequencing data of parasite DNA from the RTS, S trials, it was tested whether the vaccine allele (3D7)-specific "sieve-effect" would result in fewer vaccine-match infections in vaccinated participants. The analysis indicated a greater cumulative efficacy against vaccine-match parasites, and that 3D7 haplotype frequencies varied by study site throughout Africa [84]. The results emphasize that parasite variation can limit the efficacy of malaria vaccines, and highlight the potential for parasite variants that evade vaccines to spread.

5. Malaria vaccine pipeline in general

It was known about or more than thirty *P. falciparum* malaria-vaccine trials, which are at advanced preclinical or clinical stages trials [85], Specially, that target blood parasitic stages, in the form of recombinant protein antigens, are being developed, where as only RTS, S/AS01 (a pre-erythrocytic stage vaccine) has completed phase III evaluation and reached the regulatory review stage. Four other approaches have been tested in Phase 2b trials with several hundred volunteers each. Among these are ChAd63/MVA ME-TRAP, MSP3, GMZ2 and PfSPZ. ChAd63/MVA ME-TRAP uses two different recombinant viral vectors to induce T cell responses to the liver stage antigen TRAP. On the other side, GMZ2 is a recombinant protein approach based on a fusion of two blood stage antigens. Both the ME-TRAP and GMZ2 programmes have enrolled hundreds of volunteers in multiple trials across Africa. MSP3, another blood stage antigen, has mainly been tested in Mali. Whole parasite vaccines are under development. In one of these vaccines, known as PfSPZ, sporozoites are attenuated by irradiation while still in the mosquito's salivary gland and there is subsequent extraction of irradiated sporozoites by dissection of the salivary glands of these irradiated mosquitoes. Other whole-

organism approaches to malaria immunization are being explored using various methods, including genetic attenuation of sporozoites. In addition to the approaches outlined above, there are many others in clinical evaluation or at an advanced stage of pre-clinical evaluation [86].

The most advanced candidate is the vaccine against *P. falciparum* malaria disease known as RTS, S/AS01, which is based on the *P. falciparum* sporozoite antigen CSP, was developed after a series of clinical trials demonstrated that simpler CSP-based vaccines. Furthermore, in addition to using a novel delivery system based on the hepatitis B–malaria antigen fusion protein, novel adjuvants have been utilized because RTS, S formulated on aluminium-containing adjuvants alone afforded no protection in human-challenge studies [87]. Various RTS, S/adjuvant formulations have been compared in human-challenge studies, and the formulation designated as RTS, S/AS01 appeared to provide the greatest protection [89].

As RTS, S/AS01 only contains CSP malaria antigen, the only possible biological action of the vaccine is at points 1 and 2 in figure 2. 2. These results in either completely prevent an incident liver-stage infection, reducing the numbers of sporozoites infecting hepatocytes after an infective bite, or inhibiting liver-stage development completely or partially. CSP is not expressed in the blood stage, and so RTS, S/AS01 immune responses do not directly affect the blood stages of the life cycle [88].

6. RTS, S Immunogenicity

6.1. Theoretical mechanism of action of RTS, S

It is well described that RTS, S/AS reduces the rate of acquisition of new blood stage infections (initial inoculum) of plasmodium and down grade the multiplicity of infections in vaccines. This might result from the induction of CS-specific antibodies and/or CD4+ T cells [89].

 immunogenic [90, 91], although some sporozoites may perhaps entering directly into vessels during mosquito probing. Anti-CS antibodies have been shown to reduce the numbers of sporozoites that enter skin blood vessels to begin the journey to the liver [91]. No anti-CS antibody threshold level has been found as indicative of full protection against infection: the data are consistent with a dose response such that at higher IgG concentrations a reduced risk of infection is seen. Importantly, antibody titres after the fourth dose do not reach levels seen after the first three doses, which is consistent with efficacy also not being as high. The reasons for this are not fully understood. One hypothesis is that high titre hepatitis B antibodies induced by first three doses would interfere with subsequent induction of anti-CS immunogenicity. A more likely

hypothesis, supported by the lower anti-CS titers elicited in malaria immune than naïve adults [92], is that increasing exposure to CS – whether through repeated malaria infection or vaccination - leads to B cell hypo responsiveness. This phenomenon, first described for meningococcal and pneumococcal polysaccharide vaccines [93], reflects the recruitment and differentiation of fewer antigen-specific B cells into successive responses, the B cell reservoir being exhausted by repeat and/or high-dose antigen exposure [93].
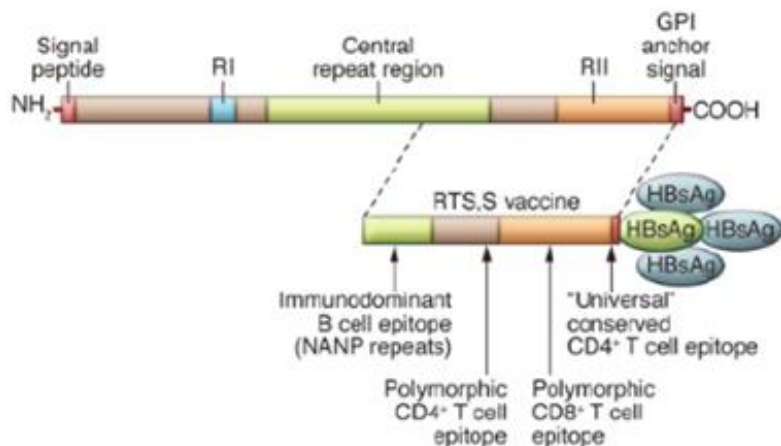

Figure-3. Schematic representation of the CSP and the RTS, S vaccine- (peter *etal*. 2010)

Cell-mediated immunity (CMI) indicators were used as a down-selection criterion for adjuvant choice in the RTS, S development program [91]. Both CS-specific γ-interferon secreting CD4+ T cell responses and multifunctional CS-specific CD4+ T cells (defined as expressing two or more of γ-interferon, TNF, IL-2 and CD40 ligand using an intracellular cytokine staining assay) are important in protecting vaccines of RTS, S [88]. Multifunctional CD4+ T cell responses were reported not to be correlated with IgG responses. Moreover, some data on CMI responses to RTS, S available in African children [88], showed an absence of substantial CS-specific CD8+ T cell responses [90]. Therefore, CD8+ T cells are thus not thought to be an important mediator of protection for RTS, S/AS01. Prior to the l Phase 3 trials, there was a consistently reported association between IgG that bind CS and protection from infection, but not from disease. This is consistent with the pre-erythrocytic biological target of the vaccine. It is possible that complete protection occurs in some volunteers, but in high transmission settings, most vaccines do eventually develop malaria, suggesting that the proportion completely protected is, at most, small. This needs to be taken into account in interpreting associations of immune responses and efficacy, as partial protection from infection might be expected in most individuals. This also

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implies that vaccinated individuals, during the initial period when protected against malaria, also experience less exposure to blood-stage parasites and therefore may have a deferred development of naturally acquired immunity, which may render them later on more susceptible to adverse effects of malaria infection as vaccine efficacy wanes than persons who have not been vaccinated. When we see the immunogenicity of RTS, S/AS in Phase 2 and Phase 3 studies, other than the Pivotal Phase 3 study, in the pediatric population, after 3 doses of RTS, S/AS01 vaccine given according to the 0, 1, 2-month schedule, over 98% of subjects were seropositive for anti-CS antibody response. Seropositivity was defined as 0. 5 EU/ml. Immunogenicity tends to increase with decreasing age from adulthood to a peak at median age of 11-12 months. From the age of 11-12 months, a decrease in immunogenicity with age de-escalation to infants vaccinated at 1-2 months of age is seen [90, 91, 93].

Adenovirus (Ad35) vectored CS RTS, S/AS01E induced very potent anti-CS Ig responses, modest CD4+ g-interferon T cell responses and low or absent CD8 responses. The approach considered most likely to improve upon CS-mediated protection would be to employ a prime-boost combination of RTS, S/AS01 with a CD8-inducing CS vaccine. The non-replicating adenovirus 35 vector encodes the CS protein. In preclinical development, the vaccine induced strong IFN-g responses in mice including CD8+ responses, thought to be important for protective immunity in humans [93, 94]. Phase 1 human studies examining safety and immunogenicity have occurred at Stanford and Vanderbilt Universities [95] with a Phase 1b study in Burkina Faso now completed [96].

6.2. RTS, S/AS01 Vaccine Safety

An ART, S/AS01 is a solely new malarial vaccine, and AS01 has not yet been used in other licensed vaccines. There is clinical experience with AS01 in a number of other non-malaria experimental products, including in adults in a Phase III trial of varicella–zoster virus glycoprotein E and AS01 [94]. And after nearly 12, 500 infants and children have received the RTS, S/AS01 vaccine in clinical trials, the WHO Global Advisory Committee on Vaccine Safety (GACVS) reviewed the safety data for RTS, S/AS01 in 2009, 2014 and 2015 and determined the acceptable safety profile RTS, S/AS01 [97, 98, 99].

7. Challenges of malaria vaccine development

7.1. Challenge related to the parasite

The main difficulties were the malaria parasite's (P. falciparum) extremely complex biology, life

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Cycle, genetic diversity and parasite's evasion and strategy of the human immune system

Because of the high number of polymorphisms or allele-specific variations in the proteins, single protein-based vaccines had limited success. The Plasmodium parasites' genetic make-up consists of about 5400 coding genes, and with the absence of adequate natural human immunity against the disease, these make malaria unique from other microbial pathogens for which successful vaccines have been developed [100.101, 102]. Moreover, malaria has been mutating for many million years, and after a person has contracted malaria, they can only acquire partial immunity—unlike a virus which can elicit ful immunity so different immune system arms are required depending on the parasite's extracellular or intracellular location and distinct immunogenic properties. The protective antibodies against sporozoites (sexual forms transmitted by the mosquito in man) fail to recognize merozoites (asexual erythrocytic stages that cause clinical malaria). This means that if only one sporozoite evades the antibodies released as response to a vaccine, we can expect approximately 10,000–40,000 merozoites to be active after one week to start clinical disease. This poses a big challenge to developing a highly effective vaccine to malaria [103, 104].

7.2. Challenges in selecting suitable malaria vaccine candidates

Identification of malaria vaccine antigens, understanding of the pathogen disease mechanism, construction DNA that will express proposal properly folded functional protein and host immune response interactions have been a major challenge. This is due to the complex life cycle of the plasmodium parasite and P. falciparum has demonstrated the capability, through the development of multiple drug-resistant parasites, for evolutionary change. The Plasmodium species has a very high rate of replication, much higher than that actually needed to ensure transmission in the parasite's life cycle. This enables vaccine trials and development. However, such antigens are most often polymorphic, and even exhibit clonal variation through differential multigene expression. Despite these challenges, efforts have been made to develop malaria vaccine candidates [105] and a great achievement in vaccine development has advanced to the clinical phase. However, this may also show inability to predict protection induced by a particular candidate vaccine in early phases [106].

7.3. Challenges in the implementation plan for vaccine trials

A vaccine is given in order to induce the adaptive immune responses to against a particular pathogen. Regardless of the success in the development of malaria vaccines, there is still a lack

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of understanding of individual immunity against malaria. Since the work of Koch on Java Island at the end of the 19th Century, which showed that adults who survive malaria infection acquire a highly effective immunity, the mechanisms involved and how they operate remains partly unknown, although the antibody that blocks the invasion of merozoites into erythrocytes appears to play a crucial role [107]. Some work has been done to determine which protective antigens or epitopes can be used in the construction of recombinant, subunit or synthetic malaria vaccines [108, 109]. Surrogate markers of antibody efficacy currently rely on in vitro assays that are difficult to reproduce, and it remains unclear. This also associated due to the lack of suitable animal model permissive for *P. falciparum* [110]. Currently, there are no clear correlates of immunity against pre-erythrocytic and blood-stage parasites. Immuno-assays can be validated only once a vaccine demonstrates efficacy in a clinical trial. Once an immune correlate for protection is identified, it can be used for decision making in clinical development. Studies have demonstrated that immunity against blood stage *Plasmodium falciparum* is associated with the acquisition of anti-parasite antibodies of the cytophilic subclasses [111], and in particular IgG3 [110, 111]. Recently, it has been shown that, there is an association between the frequencies of RTS; S/AS01E induced (circumsporozoite protein) CSP-specific CD4+ T cells and protection from clinical malaria, most strongly seen for IFNc-IL2-TNF + CD4+ T cells. Furthermore, there were significant interactions between CSP-specific TNF + CD4+ T cell responses and anti-CSP antibodies induced by RTS, S/AS01E vaccination. This interaction suggests that the protection afforded by the combination of CD4+T cells and anti-CSP antibodies is greater than would be predicted by their sum. RTS, S vaccine candidate induces high concentrations and frequencies of antibodies and CD4+ T cells, respectively, specific for circumsporozoite protein (CSP) [112, 113]. Anti-CSP antibodies correlate with protection against infection in malaria naïve adult challenge studies [112] and field studies in young children [113], against clinical malaria in trials with young children [114] and but anti-CSP antibodies did not correlate with protection against clinical malaria in a trial with older children [112, 114]. Anti-CSP antibodies could protect by a variety of mechanisms including complement activation, antibody dependent cellular cytotoxicity, sporozoite neutralization, and/or FccR mediated phagocytosis [113].

7.4. Follow-up and adherence to the study protocol

Adherence to the study protocol in clinical trials is an important prerequisite for both the investigators and the study participants. Failure of safety evaluation by the investigators or loss

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of follow-up among study participants obviously creates major setbacks in research findings. The reason to failure of follow-up can be (death, illness, worsened health, and refusal, withdrawal, side effects and general dissatisfaction by participants with regard to trial conduct) [115]. A well-planned and conducted informed consent at individual and community level can substantially reduce withdrawals and losses to follow up [116]. Participants may not comprehend all the information provided in the informed consent due to the poor consenting process or literacy status of the participant or misinformation about research in the community. Engaging the community and participants is cited as an element for a successful retention of participants in a study. Modern technology has helped to overcome poor infrastructure in African trial settings. Participants can now be reached through mobile phones to enable research teams to ask parents the health status of their children, remind them of pending visits and schedule emergency care. The conduct of clinical trials is complex - not only in terms of products being tested, but also in balancing scientific and ethical obligations to study populations. The capacity to ethically review study protocols and provide ethical oversight of clinical trials is a core component of responsible research systems. Besides the elements of human, physical and social (community involvement) capacity building, sustainability of a trial center will only be assured once the center develops a full trial portfolio that goes beyond a single disease aspect and single disease research programs [117].

7.5. State-of-the-art facilities for clinical care, laboratories, expertise

The criteria for selection of a promising vaccine will provide a clear pathway and promote greater confidence among scientists and funders that investments are focused on the best candidates. This approach could facilitate collaboration between African scientists and pharmaceutical companies in the formulation and development of vaccine candidates. Exchange of expertise will allow African investigators to be involved in protocol development and study design, eventually building confidence and the basis to participate in phase I, which is usually conducted in developed countries. These research-training programs are aimed at developing sustainable partnerships with select scientific institutions in emerging countries to further this endeavor [118]. The establishment of phase I and challenge units in malaria endemic countries will expedite product development and allow screening of products early in the target population allowing for optimization of the candidates before the conduct of field trials. Additionally, researchers conducting clinical trials in developing countries have ethical

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obligations beyond those falling on researchers working in the developed world, such as ensuring access to standards of care and ensuring the rights of study participants are held as the oversight systems are not well developed. No single profession, team, or country has a monopoly on wisdom. Establishing partnerships will assure procedural fairness and promote the ethical conduct of clinical trials in a world characterized by grave inequities [118].

7.6. Challenges facing particularly RTS, S

The regulatory pathway for vaccines is complex, and vaccine programs are highly sensitive to public perceptions of safety. A recommendation for use in country national programs may require additional data that could result in changes to the clinical development plan currently in place. The introduction of RTS, S is based on an expectation of using existing national immunization programs. However, history has taught us that introduction of new vaccines takes years, and as long as a decade or more. Finally, several African countries appear to have controlled or are close to controlling malaria, and may question the additive impact of a vaccine. In other places, clinical malaria might be observed in older age groups for which a pediatric vaccine is not indicated. Questions have also been raised related on the cost-effectiveness of a partially effective vaccine [119].

8. Opportunity of malaria vaccine development

8.1. Funding

Funding has become increased substantially over the last years. Contributions from different agencies like the Bill and Melinda Gates Foundation (particularly through PATH Malaria Vaccine Initiative-non profitable organization), the US National Institute for Allergy and Infectious Disease, European Union DG RTD, United States Agency for International Development, Well come Trust, Medical Research Council UK, the European Vaccine Initiative (formerly EMVI), European and Developing Countries Clinical Trials Partnership and WHO could be taken as opportunities for fighting malaria in particular, infectious diseases as a whole [120].

8.2. Access to technology

Much possibility is there to prioritize cost-efficient delivery and adjuvant platforms for which significant safety databases are available from developed world products and even there is an opportunity to develop TPPs with input from key stakeholders, including national authorities in malaria endemic countries. The other is the presence of available opportunity in strengthening of

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the routine surveillance systems in developing countries and the establishment of a network of pharmacovigilance sentinels sites, including via the Global Vaccine Safety Initiative. The access to frame work the development of panels of monoclonal antibodies, preferably with functional activity, to support antigenicity assessment, prior to advancing to time-consuming preclinical immunogenicity studies could also be other fertile conditions [121].

9. Conclusion

Along period trials and effort to develop an efficacious vaccine as an option to doom malaria related morbidity and mortality have been challenged because of complicated life cycle of malarial parasite, its genetic diversity, and limited access to technology in edemic area development. However the more adanced and first-ever malaria vaccine, RTS, S AS01 was approved for widespread use on 2021. It is clear that further advances are still required for malaria further vaccines development, based on empirical approaches and basic research by identifying new target antigens and taking advantage of new technologies and strategies, which will speed up the development of highly efficacious new generation of malaria vaccines.

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