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Host-parasite interactions during *Plasmodium* infection: Implications for immunotherapies

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Malaria is a global infectious disease that remains a leading cause of morbidity and mortality in the developing world. Multiple environmental and host and parasite factors govern the clinical outcomes of malaria. The host immune response against the *Plasmodium* parasite is heterogenous and stage-specific both in the human host and mosquito vector. The *Plasmodium* parasite virulence is predominantly associated with its ability to evade the host's immune response. Despite the availability of drug-based therapies, *Plasmodium* parasites can acquire drug resistance due to high antigenic variations and allelic polymorphisms. The lack of licensed vaccines against *Plasmodium* infection necessitates the development of effective, safe and successful therapeutics. To design an effective vaccine, it is important to study the immune evasion strategies and stage-specific *Plasmodium* proteins, which are targets of the host immune response. This review provides an overview of the host immune defense mechanisms and parasite immune evasion strategies during *Plasmodium* infection. Furthermore, we also summarize and discuss the current progress in various anti-malarial vaccine approaches, along with antibody-based therapy involving monoclonal antibodies, and research advancements in host-directed therapy, which can together open new avenues for developing novel immunotherapies against malaria infection and transmission.

KEYWORDS
Plasmodium, immune evasion, immunotherapeutics, vaccine candidates, antibody therapy, host-directed therapy

1 Introduction

Plasmodium is a genus of unicellular eukaryotes that are obligate parasites of vertebrates and insects. Protozoan parasites belonging to the genus *Plasmodium*, mainly cause malaria, which is prevalent mainly in tropical and subtropical regions, and is a major global health problem (1). Malaria is a life-threatening disease, which is

transmitted to humans *via* the female *Anopheles* mosquito. Although there are more than 100 species of *Plasmodium* which can infect many animal species, five species of *Plasmodium* (*P. falciparum, P. vivax, P. malariae*, *P. ovale* and *P. knowlesi*) have long been recognized to infect humans and cause illness (2). Among these five *Plasmodium* species, infection with *P. falciparum* accounts for more than 90% of the world's malaria mortality and *P. falciparum* and *P. vivax* are involved in causing high disease burden in Sub-Saharan and Asian regions (3, 4). According to the latest statistics, there were approximately 241 million cases of malaria globally with nearly 627,000 deaths in 2020 (5). A high incidence of malaria has been reported in the African region which contributes to about 95% of cases resulting in 96% of malaria deaths; out of which, children under the age of five accounted for 80% of malaria deaths (5). *Plasmodium* life cycle alternates between the primary host (mosquito) and secondary host (human). *Plasmodium* completes asexual development inside human hepatocytes and erythrocytes. Inside hepatocytes, the parasite undergoes differentiation into trophozoite and schizont stages to form first generation of merozoites (6). Merozoites invade human red blood cells (RBCs) and undergo erythrocytic schizogony to develop through ring, trophozoite and schizont stages. Schizonts release merozoites that continue to infect erythrocytes to initiate the erythrocytic cycle. Some of the asexually replicating parasites commit and differentiate into gametocytes. Gametocytes develop through stages I-V over two weeks inside erythrocytes and erythroblasts (7). Stage V gametocytes are taken up in blood meal and they rapidly differentiate into gametes. A male gametocyte undergoes three rounds of rapid DNA replication to form eight flagellated male gametes (microgametes). On the other hand, a female gametocyte forms a single female gamete (macrogamete). Male and female gametes undergo fertilization to form a short-lived zygote. This short-lived zygote differentiates into motile ookinete. The ookinete ultimately develops into oocysts. Sporozoites forms inside oocysts which migrate to the salivary glands of mosquito. Sporozoites stay in the salivary glands for initiation of the next infection cycle (6, 7). The *Plasmodium* life-cycle thus represents a series of differentiation stages, which are characterized by the expression of stage-specific proteins, some of which are targets of host immune response.

In this review, we have discussed various host defence mechanisms and counter mechanisms employed by *Plasmodium* when it undergoes multiple stages of development inside a human host. Various anti-malarial drugs, such as chloroquine and primaquine are associated with adverse side effects. Additionally, malarial parasite can acquire drug resistance, which necessitates the development of alternative immunotherapeutics (8, 9). Despite numerous studies on vaccine candidates, there is no licensed vaccine against *Plasmodium* infection. The major obstacle in anti-malaria vaccine development is antigenic variants, therefore identification of promiscuous T-cell and B-cell epitopes may improve vaccine development strategies. This review provides current and updated information regarding various anti-malarial vaccine candidates. Since humoral immune responses and antibody effector functions largely contribute to anti-malaria immunity, this review also details various monoclonal antibodies developed and their efficacy against multiple stages of *Plasmodium* parasite. Furthermore, we discuss the development of host-directed therapy which can block the transmission of the parasite and may prove to be effective in the management of severe malaria infections.

2 Parasite survival or immune evasion strategies in mammalian and mosquito hosts

Although the host immune system can reduce the parasite burden, malarial parasites have a variety of efficient immune evasion mechanisms. These immune evasion mechanisms make the host immune system ineffective to prevent the parasite's development and progression through the skin, liver, blood, and spleen at various stages.

2.1 Parasite survival or immune evasion strategies in mammalian host

During vector transmission to humans, *Plasmodium* sporozoites are injected into the dermis. The sporozoites migrate from the dermis to the liver and proceed to the liver stage and blood stage cycle. *Plasmodium* parasite undergoes a complex infection cycle where it interacts with various host cells and modulates their functions (10). Early clearance of parasites by the innate immune system is inefficient due to several strategies employed by *Plasmodium* to evade the host immune system. Skin is a physical barrier that sporozoites encounter after transmission into the human host (11). Sporozoites employ strategies such as cell traversal and motility to pass this physical barrier. Cell traversal proteins such as SPECT1 (sporozoite microneme protein essential for cell traversal) and SPECT2 are utilized by sporozoites to achieve successful migration to the liver (12). Another sporozoite surface protein, TRAP (thrombospondin-related anonymous protein) is responsible for sporozoite motility through the dermis. TRAP also interacts with host cells through binding to sulfated glycoconjugates motifs which results in cell surface recognition and entry to liver cells (13). Upon mosquito bite, neutrophils are the first to be recruited at the site of infection. Neutrophils and monocytes can phagocytose sporozoites. However, upregulation of the Agaphelin protein can have negative effects on neutrophil chemotaxis and NET development (14). Monocytes can inhibit

the growth of parasites by antibody-dependent cellular inhibition (ADCI) (15). However, ingestion of hemozoin (parasite pigment) impairs the function of monocytes and macrophages and represses their ability to produce inflammatory cytokines (16).

2.1.1 Parasite survival or immune evasion strategies during the liver stage

To establish a successful infection in hepatocytes, the sporozoites need to cross the barrier of specialized phagocytic cells in the liver, also known as Kupffer cells (KCs) (17). Although KCs can kill most invading microorganisms, sporozoites have various strategies to evade KC-mediated defence response. The interactions of sporozoites are mediated by circumsporozoite protein (CSP) which binds to heparin sulfate proteoglycans present on the surface of KCs (18). CSP also interacts with LRP-1 (low-density lipoprotein-related protein), which upregulates the intracellular levels of cAMP/EPAC and prevents ROS formation. Prevention of ROS formation contributes to parasite survival (19). In the rodent malaria model involving *P. yoelii*, it has been reported that sporozoites can modulate the cytokine response *via* upregulation of Th2 cytokines and downregulation of Th1 cytokines, which aids in sporozoite survival and invasion through liver cells (20). CSP protein has been shown to inhibit IL-12, IL-6 and TNF-α secretion, and increase IL-10 and TGF-β levels, which can aid in immune invasion (21, 22). Furthermore, sporozoites can also manipulate the key functions of KCs by impairing their antigen presentation capacity and inducing forceful apoptosis (23). *Plasmodium* parasite is also known to produce MIF (macrophage inhibitory factor) cytokine. MIF inhibits the migration and activation of phagocytes. It can also manipulate T-cell differentiation resulting in reduced anti-*Plasmodium* $CD4^+$ T-cell response (24, 25).

Antibodies against free sporozoites and CSP are the first line of defence to prevent the invasion of hepatocytes (26). Antibody-effector functions such as neutralization, complement activation phagocytosis and antibody-dependent cellular cytotoxicity (ADCC) play an important role in eliminating sporozoites (27). However, the parasite can shed CSP during cell traversal in the liver and evade antibody-mediated clearance. Furthermore, CSP has multiple tandem repeats which can downregulate antibody isotype maturation. Sporozoites are known to modulate hepatocyte functions which contribute to their intra-hepatocytic proliferation and survival. Release of CSP by sporozoites causes suppression of the NF-kB signalling which negatively affects the host immune mechanisms (28). Sporozoites alter host inflammatory responses *via* upregulation of host heme oxygenase-1 protein (HO-1) (29). Furthermore, sporozoite infection of hepatocytes affects the mTOR pathway, which leads to an alteration of intracellular proteins involved in cell growth, proliferation, and survival (30). After hepatocyte invasion, sporozoites develop a membrane called parasitophorous vacuolar membrane (PVM) around their cell surface which protects them from selective autophagy and apoptosis. This membrane-enclosed structure helps the parasite to overcome its intracellular degradation while residing inside the host cells (31). A parasite-derived PVM-resident protein upregulated in infectious sporozoites 4 (UIS4), interacts with the host cell actin and by suppressing filamentous actin formation, UIS4 avoids parasite elimination (32). Hepatic Merozoites employ various immune evasion strategies to overcome the role of liver phagocytic cells in their development. Merozoites protect themselves from the liver phagocytic cells by getting released inside merosomes (33). These immune evasion strategies employed by merozoites during liver stages further clear their path for entering to blood stage. Each hepatic merozoite can subsequently invade RBCs and initiate blood stage development. Since RBCs do not express MHC molecules on their surface, erythrocytic merozoites escape recognition by $CD8^+$ T-cells (34).

2.1.2 Parasite survival or immune evasion strategies during blood stage

During the blood stage of infection, *Plasmodium* employs various immune evasion strategies to evade the host's immune response. *Plasmodium* manipulates the NF-kB and Type 1 interferon pathway to drive inflammation responsible for malaria pathogenesis (35). Intracellular parasitism is responsible for the immune escape of the parasite from antibodies. As antibodies can only bind extracellular/free sporozoites or merozoites, therefore when parasites invade host cells, antibodies cannot cross the cell membrane, preventing the antibody function (36). Antigenic diversity/polymorphism and expression of antigenic variants at different stages of infection are two major immune evasion strategies which promote parasite survival and contribute to long-lasting parasite infections (36). To invade RBCs, merozoites express a variety of surface proteins like MSP-1 (merozoite surface protein). MSP-1 interacts with glycosylphosphatidylinositol (GPI) anchors present on RBCs (37). Antigenic diversity involves the expression of antigenically different alleles of a gene in different parasite populations. For example, *msp1* has many alleles and antibodies to one *msp1* allele cannot recognize others. Another class of merozoite proteins namely erythrocyte binding-like (EBL) proteins promote immune evasion. Both MSPs and EBLs are present as multiple alleles, thereby showing a high degree of polymorphism (38, 39).

2.1.3 A mechanism of antigenic variation during blood stage

The most prominent immune escape strategy which is employed by *Plasmodium* is the expression of antigenic variants during its blood stage. Antigenic variation is maintained by variant surface antigens (VSAs). VSAs consist primarily of an immunodominant molecule known as *P. falciparum* erythrocyte

membrane protein 1 (*Pf*EMP1) encoded by the *var* multigene family (40, 41). *Pf*EMP1 protein expression on infected RBCs (iRBCs) is responsible for adhesion to endothelial cells (40). Adherence of parasitic forms to endothelial cells aid in immune evasion, preventing their entry into the spleen and liver, which may lead to severe forms of cerebral malaria (42). Antibodies to *Pf*EMP1 on the surface of iRBCs interfere with its binding to endothelial cells. Antigenic variation helps the parasite to escape the host antibody response. The genome of *P. falciparum* contains about 60 *var* genes, encoding a different variant of *Pf*EMP1. The gene expression of *Pf*EMP1 is highly regulated and only one *var* gene express at a time. Although antibody-mediated response against a single *Pf*EMP1 variant can reduce the parasite burden to some extent. However, a small fraction of parasites switch the *var* gene expression, encoding a different *Pf*EMP1 variant which results in immune evasion from antibody-mediated response (43). *Pf*EMP1 encoding region of *var* gene contains two exons and one conserved intron. Each *var* gene contains two promoters, one promotor gives rise to *Pf*EMP1-encoding mRNA which contributes to mutually exclusive expression of *Pf*EMP1 variants. The other bidirectional promotor found within the intron region drives the expression of chromatin-associated sense and anti-sense, long non-coding RNAs (lncRNAs) (44). Regulatory elements such as lncRNAs may have transcriptional control over *var* gene expression. While sense lncRNAs are expressed during later stages of parasite development, the antisense lncRNA is expressed only from the single active *var* gene at the early stages of parasite development in RBCs, when *var* mRNA is transcribed (45). Anti-sense lncRNA recruits the proteins required for chromatin modifications and transcriptional activation. They are majorly involved in the mutually exclusive expression of *Pf*EMP1 variants which contribute to antigenic variation and host immune evasion by parasite (46). Recently, one group of researchers have identified an anti-sense lncRNA-associated protein, *Pf*TPx-1 which localizes to specific nuclear subcompartment and creates a redox-controlled microenvironment essential for the active transcription of *var* genes. Furthermore, alterations in *Pf*TPx-1 expression influence both gene switching as well as transcriptional activation of *var* genes (47). Although *var* genes are involved in *Pf*EMP1 expression which is a key to parasite survival in their host, the mechanism of mutually exclusive expression of *var* genes is not completely understood. The histone modifications is involved in the epigenetic regulation of *var* gene expression (48). In a study, Volz et al. identified the role of histone methyltransferase, *Pf*SET10 in antigenic variation of malaria parasite. They concluded that *Pf*SET10 is not only required for *var* gene expression but it also plays an important role in parasite viability (49). However, more recently, Ngwa et al. reported that the disruption of *Pf*SET10 causes no effect on *var* gene expression (50). Furthermore, there is a lot of uncertainty and contradiction in the role of some histone deacetylase genes, *Pf*Sir2a and *Pf*Sir2b (51, 52). Various mechanisms such as changes in subnuclear localization and enzymatic activity of proteins involved in epigenetic regulation can be responsible for such huge differences/variations in experimental results. Therefore, it warrants considerable caution to interpret the results of such experiments. Notably, knockouts of *Pf*RecQ helicases cause dysregulation of *var* gene expression suggesting their role in *var* gene regulation (53, 54). In a recent study, CRISPR/dCas9 has been used to explore the role of other *var* gene regulatory elements. A complex of chromatin remodeler proteins, *Pf*ISWI has been identified which may have a role in transcriptional activation of *var* genes. Further, functional characterization of *Pf*ISWI may provide insights into transcription control of *var* genes (55). Future research is needed for the molecular and functional characterization of more epigenetic regulators which can reveal the underlying mechanisms of antigenic variation. Moreover, the inhibitors of epigenetic regulator can be employed as potent anti-malarial drugs (50). Apart from *Pf*EMP1, variant proteins such as RIFIN (early trophozoite) and STEVOR (mature trophozoite), belonging to other multigene families (*rif* and *stevor*) also contribute to the adherence of iRBCs to endothelial cells, leading to their sequestration in the microvascular system of host organs, preventing splenic elimination (42, 56). Both trophozoites and schizonts employ sequestration as another strategy for immune evasion. Interestingly, *Pf*EMP1 also induces direct immunosuppressive effects on various types of immune cells (57, 58). Recent studies using humanized mice demonstrated that parasites adapted to thrive in the humanized mice showed enhanced expression of specific *Pf*EMP1s such as VAR2CSA. Expression of VAR2CSA protected the parasites from macrophage phagocytosis and also reduced NK cell-mediated killing through interaction with the immune inhibitory receptor, LILRB1 (59, 60). Of note, the role of neutrophil mediated innate immune response against iRBCs has been examined in a recent study. The neutrophil expresses ICAM-1 which can interact with *Pf*EMP1 resulting in killing of iRBCs (61). Moreover, RIFIN proteins aid in host immune evasion *via* targeting LILRB1. They can inhibit the activation of LILRB1-expressing NK cells and B-cells. Further studies are required to understand the interactions between polymorphic proteins and host immune inhibitory receptors which may prove crucial for the regulation of malaria infection (62).

2.2 Parasite survival or immune evasion strategies in mosquito host

Mosquitoes become infected when they ingest human blood containing gametocytes. The gametocytes complete their maturation in the midgut lumen. The gametocytes differentiate into gametes, which undergo fertilization to form zygote. The *Plasmodium* zygote matures into an ookinete. Physical barriers such as peritrophic membrane (PM) of the midgut, acts as a first line of defense of *Anopheles* mosquito against ookinetes (63).

Ookinetes secretes chitinase enzyme which helps to clear their way through PM (64). Ookinetes are also exposed to the midgut proteases. To evade the midgut proteases, ookinetes express surface proteins P25 and P28 which plays an important role in midgut invasion (65). The most important parasite factor, P47 which is encoded by high polymorphic *Pf47* gene, is involved in mosquito immune evasion in *P. falciparum*. P47 interfere with the complement-like immune responses of mosquito (66, 67). Moreover, P47 also inhibits JNK pathway-mediated apoptosis of *P. falciparum* (68). In *P. berghei*, P47 is also essential for ookinete protection from the *Anopheles* complement-like response (68). Another parasite protein, PIMMS43 (*Plasmodium* Infection of the Mosquito Midgut Screen 43) expressed on the surface of ookinete and sporozoites is required for parasite evasion from mosquito complement-like response (69). The host-parasite interactions have immensely contributed to our understanding of parasite survival strategies and host immune evasion mechanisms. During the past few decades, most of the host immune evasion proteins such as CSP, TRAP, MSP, *Pf*EMP1, P28, P47 etc. have been assessed in experimental setting. These proteins have been assessed as potential vaccine candidates against different life stages of *Plasmodium*. A list of *Plasmodium* proteins involved in host immune evasion is presented in Table 1.

3 Host defence mechanisms against *Plasmodium* in mammalian and mosquito hosts

3.1 Host defence mechanisms against *Plasmodium* in mammalian host

3.1.1 Role of innate immunity in host defence in mammalian host

The complement system acts as the first line of defence against parasites and is considered a major player during innate immunity. Malarial parasite evades the host complement system at different stages. Surface molecules of *P. falciparum* are involved in capturing host complement regulator proteins which inhibits complement activities. It has been suggested that sporozoites are resistant to complement-mediated cell lysis (77). During the blood stage, free merozoites and intracellular schizonts bind to complement proteins which contributes to parasite survival. For instance, interaction of *Pf*92 and GAP50 proteins with complement regulator proteins, FH and FHL-1 leads to the inactivation of C3b (70–72). Additionally, knob-like protrusions of *Pf*EMP1 on the surface of iRBCs have been shown to prevent complement fixation (74). Of note, *Plasmodium* can hijack complement receptor 1 (CR1) as an entry receptor for invading RBCs using parasite ligand *Pf*Rh4 (73). Furthermore, *Pf*EMP1 variants can interact with various RBC receptors such as CR1 and alpha2-macroglobulin to mediate rosetting/rosette formation (75, 76). Rosette formation is another strategy employed by *Plasmodium* to evade the host immune response, wherein iRBCs form clusters with uninfected RBCs. It interferes with immune recognition and enhances parasite virulence (78). It has been reported that the release of complement-deposited digestive vacuoles by iRBCs leads to macrophage exhaustion. Furthermore, it can induce the lysis of adjacent RBCs and erythrophagocytosis, contributing to anaemia (79). A recent study showed that the acquisition of human plasminogen facilitates complement evasion by *Plasmodium*. It has been shown that the plasminogen promotes C3b inactivation and prevents terminal complement complex formation (80). Moreover, in severe malaria cases, *P. falciparum* inhibits the membrane attack complex which results in complement evasion (81).

3.1.2 Role of humoral immunity in host defence in mammalian host

Humoral immunity plays a crucial role against *Plasmodium*. Antibody-mediated responses largely contribute to host's anti-malarial immunity. The major antibody functional activities include ADCC, ADCI, growth inhibition and inhibition of host cell invasion (3, 82). *Plasmodium* parasite expresses a wide variety of parasitic factors/proteins at multiple stages. Antibodies targeting these parasitic factors have revealed the importance of stage-specific functional antibody responses in malaria. The antibody effector functions against *Plasmodium* may vary with parasite stage (4). Host antibodies generated against sporozoites can inhibit their motility, traversal and invasion to hepatocytes. Further, antibodies can enhance complement-mediated lysis of sporozoites and inhibition of hepatocyte traversal (26, 27). During blood stage, they promote phagocytosis and complement-mediated lysis of merozoites. Moreover, antibodies targeting merozoites can directly inhibit their invasion of RBCs. Furthermore, antibodies bind to the surface of the iRBC and promote their agglutination and phagocytosis (3, 11). Antibodies towards iRBCs can block the schizont egress, rosette formation and their sequestration to host endothelium and epithelium (11). More research on antibody-mediated effector functions can contribute to our understanding of host-parasite interaction which may improve the anti-malaria vaccine development strategies.

3.1.3 Role of cellular immunity in host defence in mammalian host

Along with phagocytic cells, NK cells are known to mediate innate immune functions by secreting IFN-γ enabling parasite clearance, and directly killing infected cells by cytotoxicity (16). Additionally, NK cells are also involved in killing *P. falciparum*-infected RBCs by producing perforins, IFN-γ and granzymes (83). *Plasmodium* is known to interact with dendritic cells (DCs) at every stage of their life cycle. DCs can phagocytose sporozoites

TABLE 1 List of *Plasmodium* proteins involved in host immune evasion.

S.N.	Accession no.	Protein name	Function	Cellular localization	Role in immune evasion/ parasite survival	Parasite life stages	Ref.
1.	PF3D7_1342500	SPECT1	Pore formation	Soluble and membrane-associated	Host cell traversal and migration to liver	Pre erythrocytic	(12)
2.	PF3D7_0408700	SPECT2	Pore formation	Soluble and Membrane-associated	Host cell traversal and migration to liver	Pre erythrocytic	(12)
3.	PF3D7_1335900	TRAP	Cell adhesion	Sporozoite plasma membrane	Sporozoite motility and host cell adhesion	Pre erythrocytic	(13)
4.	AGAP007907	Agaphelin	Anti-hemostatic, anti-inflammatory and anti-thrombotic activity	Secreted in mosquito saliva	Inhibitory effects on neutrophil chemotaxis and NET formation	Pre erythrocytic	(14)
5.	PF3D7_0304600	CSP	Sporozoite development during liver stage	Cell surface, cytoplasm, plasma membrane	Prevents ROS formation, upregulate Th2 response and downregulate Th1 response, downregulate antibody isotype maturation and suppression of the NF-kB signaling	Pre erythrocytic	(19–22, 30)
6.	PBANKA_0501200	UIS4	Sporozoite development during liver stage	Sporozoite plasma membrane	Avoids parasite elimination by suppressing actin formation	Pre erythrocytic	(32)
7.	PF3D7_0930300	MSP	RBCs invasion during blood stage	Merozoite plasma membrane	Antigenic diversity and allelic polymorphism aid in parasite survival	Erythrocytic	(38)
8.	PF3D7_1147800	EBL	Binding to erythrocyte during blood stage	Merozoite plasma membrane	Antigenic diversity and allelic polymorphism aid in parasite survival	Erythrocytic	(39)
9.	PF3D7_0300800	RIFIN	Cell adhesion	Surface of iRBCs	Antigenic variation, sequestration to microvascular system	Erythrocytic	(57)
10.	PF3D7_0101800	STEVOR	Cell adhesion	Surface of iRBCs	Antigenic variation, sequestration to microvascular system	Erythrocytic	(58)
11.	PF3D7_1200610	VAR2CSA	Host cell surface receptor binding	Surface of iRBCs	Reduced macrophage mediated phagocytosis and NK cell mediated killing	Erythrocytic	(60)
12.	PBANKA_0515000	P25	Midgut invasion	Surface of Ookinete	Evade midgut proteases mediated immune response	Mosquito stage	(65)
13.	PBANKA_0514900	P28	Midgut invasion	Surface of Ookinete	Evade midgut proteases mediated immune response	Mosquito stage	(65)
14.	PF3D7_1346800	*Pfs*47	Mosquito immune evasion	Surface of gametocyte	Evade mosquito complement response by suppressing midgut nitration, inhibit inhibits JNK pathway mediated apoptosis	Mosquito stage	(66, 67)
15.	PBANKA_1359700	*Pb*47	Required for female fertility	Surface of gametocyte	Protect the parasite from complement-like response of mosquito	Mosquito stage	(68)
16.	PF3D7_0620000	PIMMS43	Mosquito immune evasion	Surface of ookinete	Evade mosquito complement-like response	Mosquito stage	(69)
17.	PF3D7_1364100	*Pf*92	Recruits complement regulator proteins	Merozoite plasma membrane	Protect merozoites from complement mediated lysis	Erythrocytic	(70, 71)
18.	PF3D7_0918000	GAP50	Recruit complement regulator proteins	Merozoite plasma membrane	Protect merozoites from complement mediated lysis	Erythrocytic	(71, 72)
19.	PF3D7_0424200	*Pf*Rh4	RBCs invasion	Merozoite plasma membrane	Hijack CR1 to invade RBCs	Erythrocytic	(73)
20.	PF3D7_1200600	*Pf*EMP1	Cell adhesion	Surface of iRBCs	Antigenic variations, adherence to endothelial cells, induce rosette formation, prevent complement fixation and induce direct immunosuppression of immune cells	Erythrocytic	(42, 59, 74–76)

and prime antigen-specific T-cell responses (84). However, *Plasmodium* inhibits DC activation and functioning which interferes with the development of protective immune responses (85). In addition, *Plasmodium* infection can lead to reduced DC numbers due to increased DC apoptosis (86). T-cells *via* their cell surface receptors can recognize parasite-generated epitopes which interact with MHC molecules present on the cell surface of antigen-presenting cells (APCs). *P. falciparum* has been shown to inhibit the maturation of APCs, resulting in impaired T-cell responses (87). Among the $CD4^+$ T-cell population, regulatory T-cells play an important role in parasite immune responses. It has been shown that malarial parasites exhibit a novel immune mechanism *via* preferentially activating T-reg cells with enhanced suppressive activity (88). Proinflammatory cytokine response mediated by helper $CD4^+$ T-cells activates macrophages which helps to control merozoites *via* phagocytosis (89). Further, $CD4^+$ T-cells activate specific B-cell clones which contribute to antibody-mediated effector functions against merozoites (90). $CD8^+$ T-cells can kill parasite-infected hepatocytes using perforin and granzymes, through MHC I-associated recognition (83). Further, cytotoxic $CD8^+$ T-cells produce IFN-γ which plays an important role in the killing of intrahepatic sporozoites and is associated with protection from malaria (91). However, the role of $CD8^+$ T-cells in the blood stage is negligible because RBCs lack MHC molecules which prevent immune recognition of the parasite and help the parasite to escape $CD8^+$ T-cell response (3, 92). It has been speculated that *Plasmodium* utilizes a variety of cryptic T-cell epitopes to evade immune responses (93). Additionally, high levels of polymorphisms in the parasite epitopes can lead to immune evasion of the CTL response and alter memory T-cell effector functions (94).

3.2 Host defence mechanisms against *Plasmodium* in mosquito host

Complement-like or thioester-containing protein 1 (TEP1) is the major protein involved in the humoral immune response against *Plasmodium*. TEP1 gets accumulated on the ookinete surface for parasite killing and lysis. However, silencing TEP1 increases oocyst counts. Furthermore, TEP1 melanize the parasite and blocking TEP1 expression significantly reduces melanization of *Plasmodium* (95). *Plasmodium* utilizes two C-type lectins (CTL4 and CTLMA2) from the mosquito to escape from the immune system. Silencing of CTL4 and CTLMA2 in susceptible mosquitoes triggered melanization and reduced oocyst formation (96). Recently, Kolli et al. reported that glutaminyl cyclase (QC) mediated post-translational modifications of *Plasmodium* surface proteins can contribute to parasite evasion by disrupting mosquito immune responses such as melanization or hemocytes-mediated phagocytosis (97). The primary immune cells involved in mosquito innate immune response are hemocytes. Hemocytes such as prohemocytes, granulocytes, and oenocytoids are involved in various innate immune mechanisms against *Plasmodium* (98). Hemocytes along with fat bodies of hemolymph secrete immune factors which trigger secretion of antimicrobial peptides and induce phagocytosis, agglutination, melanization and encapsulation of parasites (99). Furthermore, reactive oxygen species (ROS) produced by hemocytes are also involved in mosquito immunity against *P. falciparum* (100). Mosquito midgut epithelial cells secrete immune-modulatory peroxidase (IMPer) which is crucial in the formation of dityrosine network. The dityrosine network is utilized by parasites to evade midgut immune response *via* inactivating NOS (Nitric oxide synthase) expression (101). Inside mosquito midgut, *Plasmodium* gametocytes differentiate into gametes, which fertilize to form zygote and subsequently progress to ookinetes. When ookinetes reaches to basal lamina, they differentiate into oocysts. Antibodies can prevent the *Plasmodium* development during mosquito stage by preventing gamete fusion and inducing complement-mediated killing of gametes/ookinetes. Antibodies can also prevent penetration and motility of ookinete through midgut wall and formation of oocysts (11). Oocysts mature and release sporozoites into mosquito haemocoel. A malaria scavenger-like (SR) protein is necessary for sporozoite development. Disruption of *Pb*SR protein inhibits sporozoite formation (102). Sporozoites show positive chemotaxis toward salivary glands. At this stage sporozoites uniformly express CSP proteins which are essential for salivary gland invasion (103). Sporozoites are accumulated in the salivary duct of *Anopheles* mosquito and are ready to complete the malaria transmission cycle.

4 Vaccine candidates against *Plasmodium*

Plasmodium expresses a variety of surface antigens during its developmental stages- pre-erythrocytic stage, erythrocytic stage, gametocyte/sexual stage and mosquito stage. Over the past few decades, various anti-malaria vaccine candidates have been assessed from different parasite stages (Figure 1).

4.1 Pre-erythrocytic stage vaccine candidates

When an infected mosquito bites the human host, sporozoites are injected through the skin. Sporozoites contain surface antigens which are involved in *Plasmodium* development in the human host. The sporozoite surface antigens act as putative vaccine antigens which can induce protective humoral immune responses and are currently under clinical trials (104). One of the most potent sporozoite surface proteins is CSP. CSP protein is required by *Plasmodium* during developmental stages

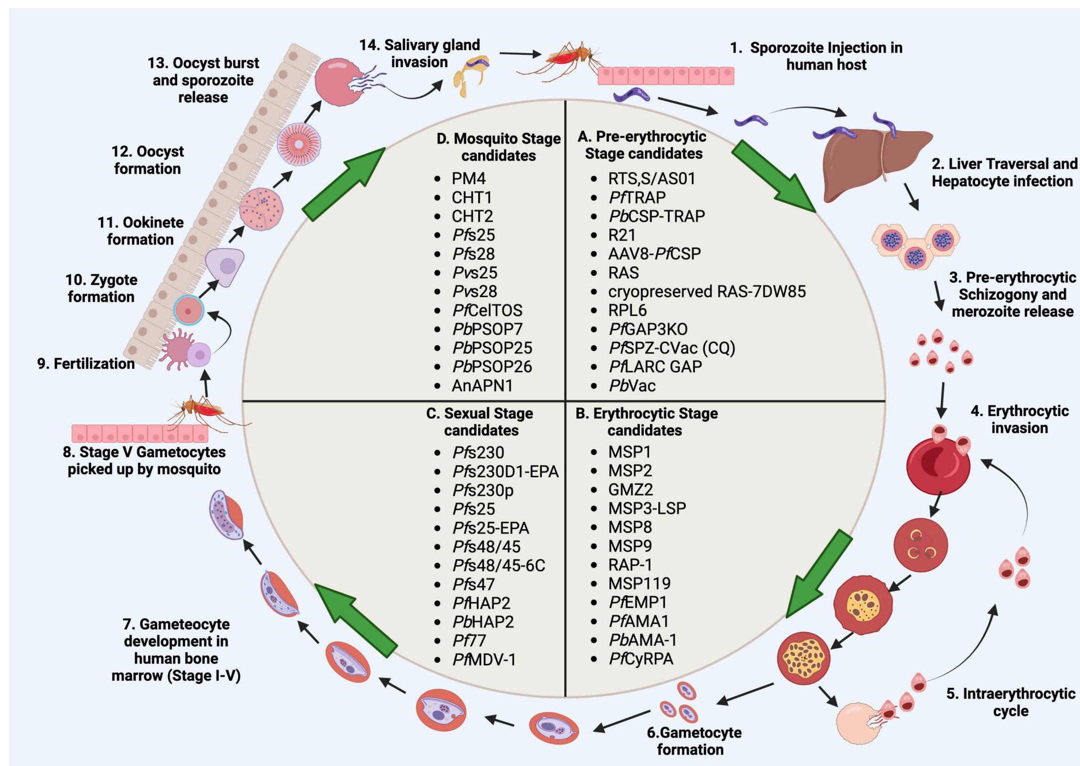

FIGURE 1
Schematic representation of malaria vaccine candidates during different developmental stages. **(A)** Pre-erythrocytic candidates (RTS,S/AS01, *Pf*TRAP, *Pb*CSP-TRAP, R21, AAV8-*Pf*CSP, RAS, cryopreserved RAS-7DW85, RPL6, *Pf*GAP3KO, *Pf*SPZ-CVac (CQ), *Pf*LARC GAP, PbVac). **(B)** Erythrocytic candidates MSP1, MSP2, GMZ2, MSP3-LSP, MSP8, MSP9, RAP-1, MSP119, *Pf*EMP1, *Pf*AMA1, *Pb*AMA-1, *Pf*CyRPA). **(C)**, Sexual stage candidates (*Pfs*230, *Pfs*230D1-EPA, *Pfs*230p, *Pfs*25, *Pfs*25-EPA, *Pfs*48/45, *Pfs*48/45-6C, *Pfs*47, *Pf*HAP2, *Pf*HAP2p, *Pb*HAP2, *Pf77*, *Pf*MDV-1). **(D)** Mosquito stage candidates PM4, CHT1, CHT2, *Pfs*25, *Pfs*28, *Pvs*25, *Pvs*28, *Pf*CelTOS, *Pb*PSOP7, *Pb*PSOP25, *Pb*PSOP26, AnAPN1). Steps 1-14 show the malaria parasite life cycle which completes in four stages; pre-erythrocytic, erythrocytic, sexual, and mosquito stages. During the pre-erythrocytic stage, sporozoites are injected by an infected mosquito into the human host which then migrates to the liver and infects hepatocytes. Sporozoites start pre-erythrocytic schizogony by forming schizonts. Schizonts rupture and release merozoites into blood circulation. Merozoites invade erythrocytes which initiates the erythrocytic stage. Merozoites differentiate into different forms such as ring, trophozoite and schizont forms. Schizonts rupture and release either merozoites or gametocytes. Merozoites start the intraerythrocytic cycle while gametocytes undergo further development in the bone marrow. While inside bone marrow, the gametocytes differentiate into sequential gametocyte stages (Stage I-V). Stage V gametocytes move to peripheral circulation and are then picked up by the mosquito. Gametocytes develop in the mosquito midgut and differentiate into microgametes (male gametes) and macrogametes (female gametes). Fertilization takes place in the mosquito midgut which forms a short-lived zygote which transforms into a motile zygote, ookinete. The ookinete develops into an oocyst and sporozoite development starts within the oocyst. The oocyst ruptures and releases the sporozoites, which then invade the salivary glands of the mosquito. The life cycle of the malaria parasite restarts when the mosquitoes bite another human host. Created with BioRender.com.

in both the primary mosquito host (mosquito stage) and secondary human host (pre-erythrocytic stage). CSP protein of *P. falciparum* sporozoites contains highly conserved protein domains structures which have been characterized by repeating amino acid, asparagine-alanine-asparagine-proline (NANP) motifs (105). CSP has been shown to induce high antibody titres indicating their role in conferring protection in animal models (106). Currently, there is only one anti-malaria vaccine which has reached phase 3 trial, namely, RTS,S, which targets *Pf*CSP protein (107). However, when RTS,S was administered with a liposome-based adjuvant, AS01, it showed limited efficacy and short-lived protection (107). RTS,S/AS01 (trade name Mosquirix) has been recently approved by WHO for broad use in children (108). Another protein antigen TRAP, which is critical for sporozoite motility is considered a promising vaccine candidate. In one study, BALB/c mice were immunized with recombinant *P. falciparum* TRAP (*Pf*TRAP) along with poly (I:C) adjuvant. Vaccination with *Pf*TRAP induced Th1 immune response and high titers of protective IgG antibodies (109). In another study, a vaccine formulation was prepared by fusion of *P. berghei* CSP and TRAP antigen along with Addavax adjuvant. The mice were immunized with *P. berghei* CSP-TRAP which elicited higher antibody titers (110). Recent studies have shown that co-immunization with several other pre-erythrocytic

vaccine antigens can confer sterile protection in rodent malaria models (111), necessitating replication of these studies using human malaria parasite pre-erythrocytic vaccine antigens. Recently, R21, a malaria vaccine, which targets *Pf*CSP has been developed. The administration of R21 with matrix M (a lipid-based adjuvant) has been shown to improve immunogenicity and enhance protection. R21 is an emerging vaccine formulation which is under phase II field trials and needs further investigations (112). In another study, intravenous administration of an Adeno-associated virus serotype 8 (AAV8) vector-based anti-sporozoite vaccine containing *Pf*CSP (AAV8-*Pf*CSP) generated protective humoral and cellular immune responses by inducing high antibody titres and recruiting liver-resident memory $CD8^+$ T (T_{RM}) cells in a mice model (113). In addition, immunization with peptides or protein fragments from a sporozoite, liver stage tryptophan-rich protein (SLTRiP) showed significant reduction in parasite numbers during liver stage by inducing a long lasting and protective $CD8^+$ T memory response (114, 115).

Intravenous administration of radiation-attenuated sporozoite (RAS) vaccines induces *Plasmodium*-specific T_{RM} cells which confer protection in mice against wild-type sporozoite challenge. RAS vaccination strategy has been improved by prime and trap strategy which involves epidermal priming of CSP antigen. A single intravenous dose of RAS aid in the activation of T_{RM} in the spleen, along with trapping and expansion of $CD8^+$ T-cells in the liver region of BALB/c mice (116). Further, cryopreserved RAS vaccination induced similar levels of $CD8^+$ T-cell responses in mouse liver and protected mice against wild-type sporozoite challenge (116). Ribosomal protein RPL6 is a natural peptide antigen which is expressed by *Plasmodium* during pre-erythrocytic stage infection. Prime and trap vaccination strategy targeting RPL6 was used for the elimination of *Plasmodium* infection in mouse liver. RPL6 induced effective protection by inducing liver T_{RM} cell response against *P. berghei* sporozoites challenge in mice (117).

Some vaccine development approaches such as genetically attenuated parasites (GAP), utilize genetic attenuation/deletion of genes essential for the completion of liver stage development (118). Sanaria® *Pf*SPZ-GA1 is a genetically attenuated whole sporozoite vaccine. It was generated by knocking out B9 and SLARP genes to halt the development of sporozoites in the early liver stages (119). Another GAP vaccine, *Pf*GAP3KO vaccine was generated by knocking out three genes, *P. falciparum p52 −/p36−/sap1−* expressed in the pre-erythrocytic stage (120). The *Pf*GAP3KO vaccine was administered to humanized mice model transplanted with human hepatocytes and RBCs. *Pf*GAP3KO was unable to complete its development from the liver stage to the blood stage, thereby protecting against the sporozoite challenge (120). Another study tested the safety and immunogenicity of the *Pf*GAP3KO vaccine in human volunteers and a single dose administration of the *Pf*GAP3KO vaccine elicited a protective antibody-mediated immune response against sporozoite infection (121). In addition, *Pf*GAP3KO protected malaria-naïve subjects from controlled human malaria infection (122). Recently, a late liver stage arresting replication-competent (*Pf*LARC) GAP was generated against the human malaria parasite. Specifically, a LARC GAP for *P. falciparum* was generated by deleting the *Mei2* (Meiosis inhibited 2) gene. The *Mei2* gene is expressed by the late liver-stage parasite. *PfMei2*$^-$ liver stages failed to complete their intra-hepatic development and do not form infectious exoerythrocytic merozoites (123). Another immunization approach which is simple, efficacious, safe and highly immunogenic during malaria vaccination is *P. falciparum* sporozoites under chemoprophylaxis vaccination (*Pf*SPZ-CVac). In this approach, human volunteers are immunized with cryopreserved *Pf*SPZ along with a 10 mg/kg chloroquine base. *Pf*SPZ-CVac immunization conferred protection in malaria-naive volunteers by inducing high levels of anti-*Pf*CSP antibodies (124). While *Pf*SPZ-CVac (CQ) was safe and conferred protection to malaria-naïve participants in controlled human malaria infection, this vaccine was unable to protect against *P. falciparum* infection in a very high transmission setting (125).

It has been shown that *P. berghei*-based vaccination (*Pb*Vac) confers cross-species protection against *P. falciparum* malaria (126). *P. berghei* is highly amenable to a genetic modification that enables the gene insertion of other human *Plasmodium* species antigens (such as CSP) into its genome loci, which may aid in the expression of heterologous *Plasmodium* antigens (127). Immunization with such chimeric *P. berghei* sporozoites derived from heterologous immunogens is expected to elicit both cross-species immune responses as well as targeted immunity against human *Plasmodium* parasites (128). *P. berghei*-based vaccines expressing both the protein, *Pb*CSP and *Pf*CSP at the surface of sporozoites were administered in rabbits *via* bites of *Pb*Vac-infected mosquitoes. This immunization elicited *Pf*CSP-specific immune responses which inhibited both *in vitro* and *in vivo P. falciparum* infection of human hepatocytes (128). Although *Pb*Vac was not able to confer sterile protection in phase 1/2a clinical trials, it elicited dose-dependent humoral and cellular immune responses, thereby reducing the liver parasite burden (129). Further exploration is required for the assessment of such vaccination approaches against *P. falciparum* malaria.

4.2 Erythrocytic stage vaccine candidates

Induction of protective humoral, as well as cellular immune responses against *Plasmodium*, is the primary goal in the development of malaria vaccines. The vaccine antigens from the erythrocytic stage can be utilized in reducing the parasite burden. The protective antibodies generated against these antigens can either block the merozoite invasion of

erythrocytes or lead to phagocytosis of merozoites (130). A variety of MSPs and invasion complex proteins are responsible for erythrocyte invasion. It has been reported that *msp1* and *msp2* show high levels of genetic polymorphism which may complicate the malaria vaccine development (131). However, another study reported that MSP1 contains conserved B-cell epitopes indicating that MSP1 could serve as a promising vaccine candidate against *P. vivax* malaria (132). In another study, the engraftment of MSP2 proteins obtained from *P. falciparum* with liposomes and supplemented with TLR4/2 antigen resulted in a strong immune response in a murine model. Briefly, immunization of mice with this MSP2 vaccine formulation generated a protective antibody response against conserved C-terminal domains of MSP2 (133). Among MSPs, the MSP3 antigen has been reported as a highly immunogenic vaccine candidate which can induce protective immune responses. MSP3 vaccine formulations such as GMZ2 (a recombinant protein fusion of GLURP (Glutamate-rich protein) and MSP3) and MSP3-LSP (a combination of MSP3 and LSP1 (Long synthetic peptide)) are under phase II clinical trials (134, 135).

VLP (virus-like particles) based vaccination strategies are considered an efficacious vaccine delivery platform for multiple antigens. Three VLPs, MSP8, MSP9 and RAP1 (Rhoptry-associated protein) were complexed with influenza virus matrix protein. Mice were immunized with a mixture of these VLPs and challenged with *P. berghei* infection later (136). VLP vaccination induced protective CD4$^+$ and CD8$^+$ T-cell responses and alleviated TNF-α and IFN-γ levels in mice sera and spleen. VLP vaccination enhanced the mice survival rate and reduced the parasite burden in peripheral blood (136). Based on genetic diversity analysis, low genetic diversity and highly conserved sequences have been reported in *P. vivax* leading vaccine candidate antigen MSP119. It has been speculated that MSP119 could be used in multivalent vaccine formulations against *P. vivax* infection (137). Another candidate malaria vaccine antigen AMA1 (apical membrane antigen) is expressed on the merozoite cell surface. *P. falciparum* AMA1 shows a high level of genetic polymorphism. To reduce the genetic polymorphism, three diversity-covering (DiCo) protein sequences were designed. Administration of *Pf*AMA1-DiCo along with Alhydrogel to malaria-exposed adults resulted in a significantly higher antibody response against DiCo variants (138). Although vaccine antigens from *Plasmodium* species have been used in generating a variety of vaccine formulations, there is no vaccine against *P. knowlesi* to date. In a recent study, using bioinformatic analysis, two potential immunogenic B-cell and T-cell epitopes of *Pf*AMA1 protein were reported, which could be used in the development of multi-epitope-based vaccines against *P. knowlesi* infection (139). In a recent study, using a heterologous prime-boost immunization strategy, three vaccine formulations namely recombinant baculovirus, VLP and recombinant vaccinia virus, each of them expressing *P. berghei* AMA1 protein were prepared. The sequential administration of these vaccine formulations in a mice model induced protective IgG antibodies and CD4$^+$ and CD8$^+$ T-cell immune responses against *P. berghei* infection providing evidence for the implementation of AMA1-based vaccination approaches (140).

4.3 Sexual stage vaccine candidates

Some of the asexually replicating merozoites commit and differentiate into gametocytes which initiate the sexual stage of *Plasmodium*. Several parasite proteins are expressed exclusively by gametocytes and constitute targets for malaria transmission-blocking vaccines (TBVs) (141). These candidates elicit human antibodies that inhibit the development of *Plasmodium* in mosquitoes, thereby preventing its further transmission. There are several TBV antigens which includes *Pf*s230, *Pf*s230p, *Pf*s25, *Pf*s48/45, *Pf*s47, HAP2 and HAP2p, *Pf*77, and *Pf*MDV-1 (141). Among TBV vaccine candidates, only two candidates: *Pf*s230 and *Pf*s25 have reached Phase 1/2 clinical trials. *Pf*s25 and *Pf*s230 are gametocyte surface proteins expressed by *P. falciparum* during the sexual stage. These proteins are essential for gamete fertility. *Pf*s25 is a female-specific protein while *Pf*s230 is expressed by both male and female gametocytes/gametes. *Pf*s230p is a paralog of *Pf*s230. *Pf*230p plays a crucial role in *P. falciparum* male fertility and zygote formation and can be investigated further as a TBV candidate (142). The administration of Exoprotein A (EPA) and *Pf*s25 conjugated vaccine in Alhydrogel®, was reported safe and immunogenic in Malian adults which induced significant serum activity after four doses. In a laboratory assay, serum activity was assessed in reducing parasite transmission to mosquitoes. However, transmission-blocking activity was not enough, and *Pf*s25-specific antibody titers declined rapidly with time (143). The effect of ALFQ, a liposomal adjuvant, on the immunogenicity of *Pf*s230D1-EPA and *Pf*s25-EPA was assessed in a Rhesus macaque model. Both vaccine conjugates generated strong antibody responses after two vaccinations. Although functional activity declined rapidly, a third vaccination of *Pf*s230D1-EPA induced functional activity which lasted for a few months (144).

In a recent clinical trial, a vaccine formulation was prepared by conjugating *Pf*s230 or *Pf*s25 antigens with EPA along with Alhydrogel. As compared to *Pf*s25, the *Pf*s230 vaccine induced a much greater complement-dependent transmission-blocking activity in humans (145). Furthermore, the limited polymorphism in P230 and conservation of sequence among *Pf*230 and *Pv*230 may aid in the development of a TBV vaccine against *P. vivax* (146). *Pf*s48/45, a cysteine-rich *P. falciparum* sexual stage surface protein is a leading clinical TBV candidate antigen (147). *Pf*s48/45 protein contains multiple disulfide bonds which are critical for its proper folding and induction of transmission-blocking antibodies. *Pf*s48/45 antigen is recognized by the most potent transmission-blocking

monoclonal antibody. The functional conservation of P48/45 in *P. berghei and P. vivax* may provide an effective *in vivo* model to test *P. vivax*-based TBVs (148). However, clinical development of *Pf*s48/45 antigens as a vaccine candidate has been hindered, due to its poor biochemical characteristics. In a recent study, bioinformatics approaches has been used to design nanoparticle-based, stabilized *Pf*s48/45 vaccines which were then administered in mice model. These multimeric *Pf*s48/45-6C vaccines elicited antibodies that drive potent transmission-reducing activity (149). *P. falciparum* protein, P47 is a paralog of *Pf*s48/45. *Pf*s47 plays an important role in protecting ookinetes from mosquito's immune system, *Pf*s47 could be a potential TBV candidate (93). The Hapless 2 (HAP2) family of proteins play a critical role in gamete fusion, and immunization with protein fragments of *Pf*HAP2, *Pf*HAP2p and *Pb*HAP2 generated transmission-blocking activity (150, 151). Recombinant *Pb*HAP2 protein administered in rabbits showed high immunogenicity by inducing HAP2-specific antibodies which inhibited *in vitro* ookinete formation and oocyst formation in *Anopheles* midgut (151). Targeting conserved fusion loops of HAP2 inhibits transmission of *P. berghei* and *P. falciparum*, which offers an opportunity for designing effective TBV vaccines (152). Other TBV vaccine candidates, such as *Pf*77 and male development gene 1 (*Pf*MDV-1) induce antibodies which show transmission-reducing activity against *Plasmodium*. Both *Pf*77 and *Pf*MDV-1 display less antigenic polymorphism and are known to induce naturally occurring antibodies in individuals living in endemic areas of Africa. These antigens are highly immunogenic and can induce transmission-reducing antibodies which may aid in the reduction of oocyst counts in *Anopheles* mosquito midgut (153).

4.4 Mosquito stage vaccine candidates

Inside the mosquito, *Plasmodium* ookinetes invade the midgut epithelium of mosquito host to transform into oocysts. During this stage, ookinetes encounter multiple barriers such as extracellular matrix (ECM) and innate immune responses of the mosquito midgut. There are some protein antigens such as PM4 (aspartic protease plasmepsin 4) and CHT1/CHT2 (chitinase) which may prove to be transmission-blocking targets of *Plasmodium* ookinete. Antibodies against both PM4 and CHT1 block the passage of ookinetes through ECM, thereby reducing oocyst counts and infectivity of malaria (154, 155). Further, *P. berghei* ookinete surface proteins such as P25 and P28 contribute to midgut invasion. Antibodies targeting proteins P25 and P28 have been shown to affect oocyst formation (156).

The most potent TBV antigens *Pfs*25 and *Pfs*28 are expressed on the surface of ookinetes (157). Both *Pfs*28 and Pfs25 have limited antigen diversity, are immunogenic and show structural similarities. It has been reported that *Pfs*28-specific antibodies can block *P. falciparum* transmission and also show synergism in blocking transmission when combined with *Pfs*25-specific antibodies. Therefore, *Pfs*28 and *Pfs*25 may prove to be effective TBV (158). A *Pfs*25-EPA-based TBV vaccine formulated with alum has been tested in adults in a phase I trial in the USA recently. Although the vaccine was safe and well-tolerated, the functional activity of the anti-*Pfs*25 antibodies was less and reduced rapidly (159). Furthermore, *P. vivax* TBV antigens, *Pvs*25 and *Pvs*28 have been reported to induce anti-parasite response and antibodies generated against *Pvs*25 and *Pvs*28 were able to completely block the *P. vivax* infection in mosquitoes (160). Another class of *P. berghei*-secreted ookinete proteins, *Pb*PSOP7, *Pb*PSOP25, and *Pb*PSOP26 show transmission-blocking activity. Mice immunization with recombinant *Pb*PSOP7, *Pb*PSOP25, and *Pb*PSOP26 proteins induced specific antibodies which recognized the ookinete surface, and mosquitoes fed on these immunized mice showed transmission-reducing activity (161). Vaccination of mice with recombinant *P. falciparum* cell-traversal protein for ookinetes and sporozoites, *Pf*CelTOS (a *P. falciparum* TBV candidate) along with TLR-based adjuvant, elicited specific anti-*Pf*CelTOS antibody-mediated immune response, which has been shown to induce transmission-reducing activity in mosquito (162). Recently, a mosquito midgut protein, namely anopheline alanyl aminopeptidase N 1 (AnAPN1), has been shown to induce potent transmission-blocking antibodies and may prove to be a potential TBV candidate (163). Moreover, Bender et al. designed a vaccine construct, UF6B, from AnAPN1 protein. The immunogenicity of UF6B was evaluated in mice, wherein mice were immunized with UF6B along with human safe adjuvant, GLA-LSQ. Vaccination with UF6b:GLA-LSQ induced humoral immune response against a potent transmission-blocking epitope indicating that UF6b vaccine construct could be a TBV candidate for malaria elimination (163). A list of various stage specific malaria vaccine candidates is presented in Table 2.

4.5 Current vaccine approaches

Despite the availability of multiple vaccine candidates, it has been difficult to develop a highly effective vaccine against Malaria, probably due to the high polymorphism associated with proposed vaccine candidates and their limited efficacy. Novel nanoparticle-based vaccination approaches seem promising due to their safety, biocompatibility, and efficacy in generating efficient anti-malaria vaccines (164). Recently, a trimethyl chitosan-based vaccine containing multiple malaria antigens from different developmental stages was prepared by using a layer-by-layer (LbL) antigen delivery platform. LbL NP vaccine administration in mice induced the highest T-cell response against *Pf*CSP indicating that it could be a potent vaccine candidate against malaria (164). *P. falciparum* cysteine-rich protective antigen (CyRPA) is a merozoite surface antigen involved in RBC invasion. In one pre-clinical study, it was found

TABLE 2 List of vaccine candidates and their mechanisms of protection against malaria infection.

S.N	Accession no.	Vaccine candidates	Mechanism of protection	Vaccine status	Clinical Trial identifier	Parasite life stages	Ref.
1.	–	RTS,S/AS01	generated anti-CSP antibodies	phase 3 clinical trials	NCT00866619	Pre-erythrocytic	(107)
2.	PF3D7_1335900	*Pf*TRAP	Th1 and IgG response	pre-clinical	–	Pre-erythrocytic	(109)
3.	PBANKA_0403200, PBANKA_1349800	*Pb*CSP-TRAP	antibody mediated response	pre-clinical	–	Pre-erythrocytic	(110)
4.	–	R21	antibody mediated response	phase1/2 trials	NCT03896724	Pre-erythrocytic	(112)
5.	PF3D7_0304600	AAV8-*Pf*CSP	high antibody titres and T_{RM} cells	pre-clinical	–	Pre-erythrocytic	(113)
6.	–	cryopreserved RAS-7DW85	CD8+ T-cell response	pre-clinical	–	Pre-erythrocytic	(116)
7.	PBANKA_1351900	RPL6	T_{RM} cell response	pre-clinical	–	Pre-erythrocytic	(117)
8.	-	*Pf*SPZ-GA1	CD8+ T-cell response	phase 1 trial	NCT03163121	Pre-erythrocytic	(119)
9.	-	*Pf*GAP3KO	antibody mediated response	phase 1 trial	NCT02313376	Pre-erythrocytic	(121)
10.	-	*Pf*SPZ-CVac (CQ)	generated anti-CSP antibodies	phase 2 clinical trials	NCT03503058	Pre-erythrocytic	(124, 125)
11.	-	*Pb*Vac	humoral and cellular responses	phase 1/2 trial	NCT03138096	Pre-erythrocytic	(129)
12.	PF3D7_0206800	MSP2	antibody mediated response	pre-clinical	–	Erythrocytic	(133)
13.	–	GMZ2	antibody mediated response	phase 2 trial	NCT00424944	Erythrocytic	(134)
14.	PF3D7_1035400	MSP3-LSP	anti-MSP3 specific IgG1 and IgG3	phase 2 trial	NCT00452088	Erythrocytic	(135)
15.	PF3D7_0502400, PF3D7_1228600, PF3D7_0105200	VLP(MSP8, MSP9, and RAP1)	Th cell, B cell and cytokine response	pre-clinical	–	Erythrocytic	(136)
16.	PF3D7_0930300	MSP119	antibody mediated response	pre-clinical	–	Erythrocytic	(137)
17.	PF3D7_1133400	*Pf*AMA1	antibody mediated response	phase 1a/b	–	Erythrocytic	(138)
18.	PBANKA_0915000	*Pb*AMA1	humoral and cellular responses	pre-clinical	–	Erythrocytic	(140)
19.	PF3D7_1031000	*Pfs*25-EPA	antibody mediated TBA	phase 1 trial	NCT02334462	Sexual stage	(145)
20.	PF3D7_0209000	*Pfs*230D1-EPA	complement mediated TBA	phase 1 trial	NCT02334462	Sexual stage	(145)
21.	PF3D7_1346700	*Pfs*48/45-6C	antibody mediated TBA	pre-clinical	–	Sexual stage	(149)
22.	PF3D7_1014200, PBANKA_1212600	*Pf*HAP2 and *Pb*HAP2	antibody mediated TBA	pre-clinical	–	Sexual stage	(150–152)
23.	-	*Pf*77	antibody mediated TBA	pre-clinical	–	Sexual stage	(153)
24.	PF3D7_1216500	*Pf*MDV-1	antibody mediated TBA	pre-clinical	–	Sexual stage	(153)
25.	PBANKA_1034400	PM4	antibody mediated TBA	pre-clinical	–	Mosquito stage	(154)
26	PF3D7_1252200	CHT1/CHT2	antibody mediated TBA	pre-clinical	–	Mosquito stage	(155)
27.	PF3D7_1031000, PF3D7_1030900	*Pfs*25 and *Pfs*28	antibody mediated TBA	pre-clinical	–	Mosquito stage	(158)
28.	PVP01_0616100, PVX_111180	*Pvs*25 and *Pvs*28	antibody mediated TBA	pre-clinical	–	Mosquito stage	(160)
29.	PF3D7_1216600	*Pf*CelTOS	antibody mediated transmission-reducing activity	pre-clinical	–	Mosquito stage	(161)
30.	PBANKA_1353400, PBANKA_1457700, PBANKA_1457700.	*Pb*PSOP7, *Pb*PSOP25, and *Pb*PSOP26	antibody mediated transmission-reducing activity	pre-clinical	–	Mosquito stage	(162)
31.	–	AnAPN1	antibody mediated TBA	pre-clinical	–	Mosquito stage	(163)
32	–	UF6b	humoral immune response to transmission blocking epitope	pre-clinical	–	Mosquito stage	(163)

that vaccine formulation containing CyRPA along with Alhydrogel elicit neutralizing antibody and anti-parasite cytokine response in mice. Therefore, it could be a potential vaccine candidate against blood stages of *P. falciparum* infection (165). Another powerful approach, insect cell culture coupled with baculovirus expression vector systems (IC BEVS), has been utilized for high-yield expression of recombinant *Pf*CyRPA protein (166). The purified *Pf*CyRPA protein was formulated with lipid-based virosome nanoparticles and used for the immunization of rabbits. Immunization resulted in the production of anti-*Pf*CyRPA specific antibodies which inhibited the multiplication of *P. falciparum in vitro* (166).

Due to HLA polymorphism in human populations, it has been difficult to generate highly efficacious vaccines against malaria. Epitope-based vaccination approaches are more promising due to the selection of epitope regions present on antigenic molecules which may further enhance the vaccine efficacy. In one study, the VLP-based approach was used to prepare an epitope-based vaccine against the blood stage of malaria. *P. falciparum* CSP protein contains a highly vulnerable L9 epitope at N-terminus central repeat region. L9 VLP vaccination confers antibody-mediated protection against the blood stage malaria in mice (167)). Another *in silico* immunoinformatics-based study was conducted to predict T-cell and B-cell epitopes in *P. vivax* PPPK-DHPS and DHFR-TS proteins (168). Since the number of predicted promiscuous epitopes in selected proteins was higher, these predicted epitopes could be considered major vaccine targets against *P. vivax* malaria and may aid in the development of effective vaccines (168). A multi-epitope vaccine was designed against the blood stage of *P. falciparum* by selecting multiple epitopes of *P. falciparum* glutamic acid-rich protein (*Pf*GARP) protein. A total of 10 epitopes (5 B and 5 HTL epitopes) were linked by suitable linkers along with flagellin adjuvant to enhance the immunogenicity of the vaccine construct (169). While *in silico* immune simulation resulted in an elevated humoral and cellular immune response against malaria, such *in silico* studies need further *in vitro* and *in vivo* evaluations (169).

Multistage chimeric vaccine-based approaches against malaria have gained attention due to their enhanced efficacy. A vaccine candidate GMZ2.6c has been designed by genetically fusion of *Pfs*48/45-6C protein with GMZ2 (a fusion protein of GLURP and MSP-3). GMZ2.6c vaccine efficacy can be enhanced by using TLR4 agonists which have been reported to induce parasite-specific antibodies and T-cell-mediated immunity in mice models (170). Recently, one study reported that the GMZ2.6c vaccine is recognized by naturally acquired antibodies in individuals living in malaria-endemic regions of Brazil with different levels of transmission (171). Another chimeric multistage TBV, ProC6C was prepared by combining *Pfs*230-*Pfs*48/45 fusion protein with the *Pf*CSP linker sequence. The ProC6C long with adjuvant Alhydrogel was administered in mice which elicited a strong antibody response which helped in reducing transmission to mosquitoes and limited sporozoites invasion to human hepatocytes (147). VAR2CSA is considered a potential vaccine candidate against placental malaria. *P. falciparum* VAR2CSA protein binds to chondroitin sulphate-A (CSA) present on the surface of the syncytiotrophoblast of the placenta (172, 173). Two vaccine formulations based on *Pf*VAR2CSA, PAMVAC and PRIMVAC are currently in Phase I clinical trials. However, VAR2CSA shows a high level of antigenic polymorphism which is a major obstacle in the development of a vaccine against placental malaria (174).

Genetic manipulation of *Plasmodium* genes is a time-consuming process, therefore lyse-reseal erythrocytes for delivery (LyRED) of miRNA are more advanced, fast and effective methods for studying novel malaria vaccine antigens. The miRNA-based translational repression can be monitored within a few days. It can be used for the characterization and identification of malaria vaccine antigens from different developmental stages which may contribute to the development of effective subunit vaccines (175). *P. vivax* merozoites contain Duffy binding protein (*Pv*DBP) which is involved in reticulocyte invasion *via* interaction with DARC (Duffy antigen receptor for chemokines) receptors present on host reticulocytes (176, 177). Although DBP shows high levels of polymorphism, the amino-terminal cysteine-rich region II found in *Pv*DBP (*Pv*DBPII) serves as an attractive target. However, the generation of a DBP-based vaccine is still a distant dream and further investigations are required to prove its efficacy against *P. vivax* malaria (178).

Chemoprophylaxis with *P. falciparum* sporozoites (CPS) is a whole sporozoite based vaccination approach. CPS immunization has been shown to induce sterile immunity in human volunteers against pre erythrocytic stage of *P. falciparum* (179). Combination of CPS with various anti-malaria drugs has been reported to improve the efficacy of such vaccines. For instance, a single dose piperaquine-tetraphosphate (PPQ) along with CPS resulted in expansion of hepatic and splenic memory $CD8^+$ T-cells in rodent malaria model (180). The efficacy of CPS immunization has been assessed in a human liver-chimeric mice model. CPS immunization induced functional IgG antibodies against *P. falciparum* sporozoites. These functional antibodies interfered with host-parasite interaction and reduced the sporozoite traversal during liver stage (181). In experimental swiss mice, CPS immunization under chemoprophylactic cover of Artether, Mefloquine/Azithromycin, Lumifentarine, and halofantrine conferred strong and long-lasting protection against *P. yoelli* sporozoite infection (182–184). Another study identified the correlates of protection for CPS vaccination by transcriptome analysis of PBMCs from CPS immunized individuals. Various correlates of protection such as interferons, Toll-like receptor (TLR), NF-kB, and monocyte-related signatures were found associated with protection. Such transcriptional analysis of post-vaccination protection signatures may prove useful for assessing vaccine efficacy

during clinical trials (185). While RTS/S/AS01E induce moderate protection in African children, CPS immunization induced 100% sterile protection in naive adults (107, 179). Overall, whole sporozoite based alternative vaccination approaches seem promising for the development of safe, effective, and potent anti-malaria vaccines.

5 Antibody-mediated therapy

Since *Plasmodium* parasites are increasingly becoming resistant to conventional anti-malarial drug-based therapy, novel antibody-based therapies can prove beneficial to prevent malaria. Antibody based therapy are highly effective and can be used in patients, non-responsive to conventional anti-malarial drug regimens (186). Studies have shown that passively transferred antibodies reduce parasitemia associated with *Plasmodium* (187–189). Multiple antibody effector functions are involved in immunity to malaria, which includes direct inhibition or neutralization, complement fixation and activation, and opsonic phagocytosis or cellular cytotoxicity by immune cells through interactions with Fc-receptors (190, 191) (Figure 2). Protective immunity to malaria is mainly associated with IgG1 and IgG3 subclasses, with IgG2 and IgG4 being associated with a decrease in opsonization (192). Humoral immune responses attack different parasite stages, and antibody-based therapy may prevent malaria infection or transmission.

5.1 Antibodies to sporozoites

Blocking sporozoite motility, dermal exit, hepatocyte traversal, and eventual invasion of hepatocytes are only a few of the sporozoite-targeting strategies used by antibodies (4). Through the activation of the complement system, phagocytosis, and Fc-mediated innate cell activities, antibodies also assist in the killing of sporozoites (191). Some mechanisms such as *in vitro* parasite neutralisation and *in vivo* protection are employed by monoclonal antibodies against the *Pf*CSP (193). Also, monoclonal antibodies against the repeat region have been shown to inhibit sporozoites

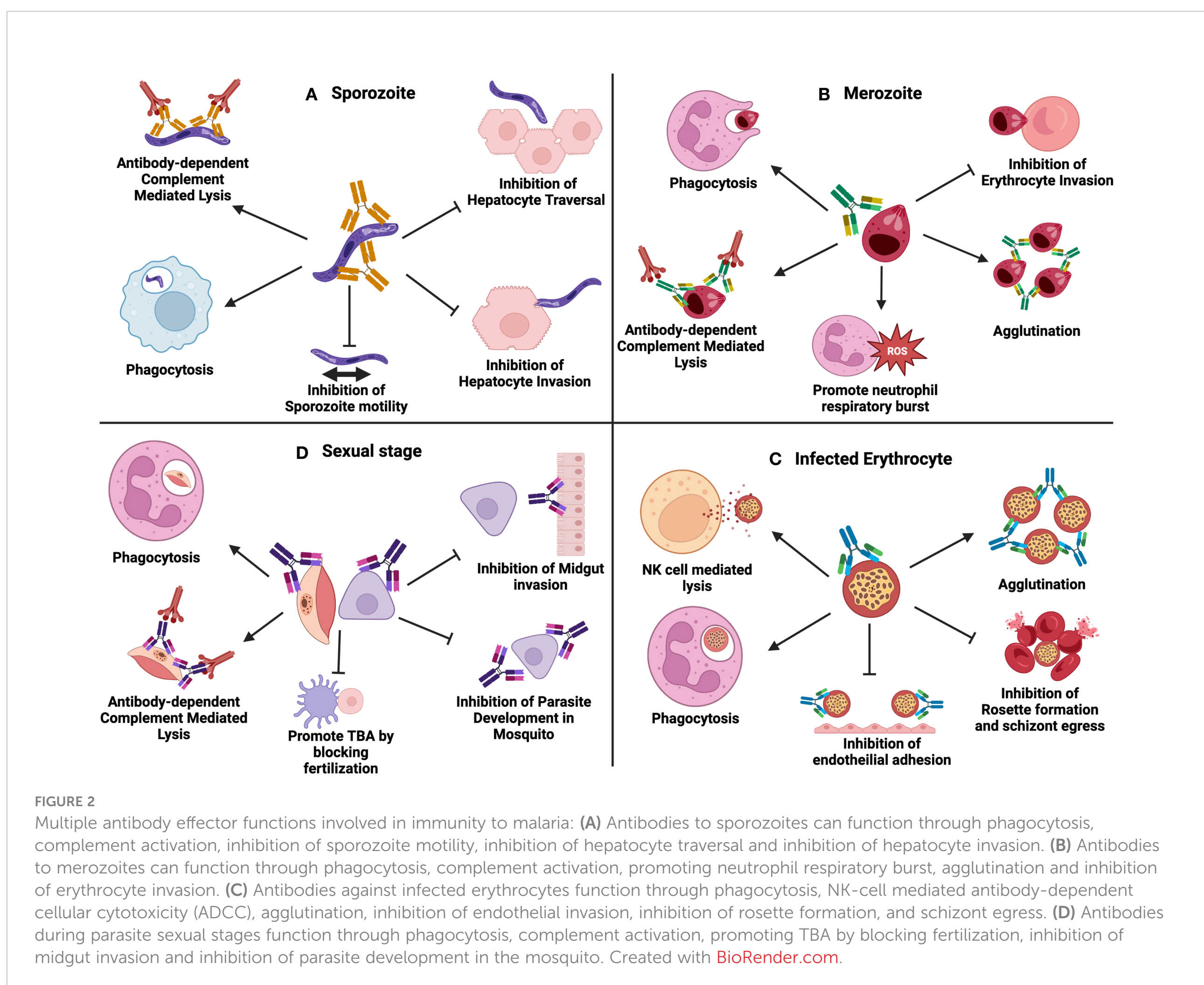

FIGURE 2
Multiple antibody effector functions involved in immunity to malaria: **(A)** Antibodies to sporozoites can function through phagocytosis, complement activation, inhibition of sporozoite motility, inhibition of hepatocyte traversal and inhibition of hepatocyte invasion. **(B)** Antibodies to merozoites can function through phagocytosis, complement activation, promoting neutrophil respiratory burst, agglutination and inhibition of erythrocyte invasion. **(C)** Antibodies against infected erythrocytes function through phagocytosis, NK-cell mediated antibody-dependent cellular cytotoxicity (ADCC), agglutination, inhibition of endothelial invasion, inhibition of rosette formation, and schizont egress. **(D)** Antibodies during parasite sexual stages function through phagocytosis, complement activation, promoting TBA by blocking fertilization, inhibition of midgut invasion and inhibition of parasite development in the mosquito. Created with BioRender.com.

(194). The testing of the CSP-based RTS,S vaccine provides the strongest support for the idea that anti-CSP antibodies can protect against malaria (195). RTS,S is a VLP consisting of a central tandem repeat of 19 NANP repeats (R) and C terminal domain of the CSP (containing T-cell epitopes) fused to the Hepatitis B Surface antigen (S). The 'RTS' fusion protein and free 'S' protein spontaneously assemble in 'RTS,S' particles. A formulation of RTS,S is undergoing Phase III clinical studies using AS01, a unique adjuvant made up of a combination of liposomes, saponin and monophosphoryl lipid A (196). Studies with RTS,S vaccine showed that antibodies can mediate sterilizing immunity, and antibodies against the sporozoite can be efficient mediators of protection against pre-erythrocytic stage malaria (197). Few human monoclonal antibodies isolated from naturally infected individuals or individuals vaccinated with RTS,S, *Pf*SPZ Vaccine, or *Pf*SPZ-CVac can inhibit sporozoite invasion in animal models (27, 198, 199). In animal models, several anti-*Pf*CSP monoclonal antibodies have been shown to be protective. Monoclonal antibodies (MAL1C, MAL2A, and MAL3B) isolated from an RTS, S-immunized individuals, imparted sterilizing immunity (197, 200). Another *Pf*CSP monoclonal antibody (2A10), isolated from the whole sporozoite immunized mice (201), was protective in vectored prophylaxis and passive infusion studies (202). Furthermore, passive transfer of a *P. yoelii* CSP monoclonal antibody (2F6) showed inhibition of liver infection when mice were challenged with sporozoites (203). Moreover, in a recent human clinical trial (Phase I) with malaria-naive volunteers, 40 mg/kg of an anti-malaria monoclonal antibody known as "CIS43LS" (directed against *Pf*CSP), was intravenously administered to patients which protected against controlled malaria challenge (204, 205). A phase I clinical trial of another CSP-specific monoclonal antibody (L9LS) was recently conducted by Wu et al. and intravenous or subcutaneous administration of L9LS, protected the recipients against malaria after controlled infection (198, 206). In addition to CSP, monoclonal antibodies against TRAP, also known as sporozoite surface protein 2 or SSP2) have been shown to prevent parasite infection of hepatocytes in both *in vitro* and *in vivo* models (207). Although people who have higher levels of antibodies against sporozoite antigens are better protected against infection, studies on the malaria vaccine have generally had unsatisfactory results using antibody titers as correlates of protection (208). The limited effectiveness of RTS,S in areas where malaria is endemic, indicates that the functioning and avidity of the antibodies, rather than the antibody titers, are better correlates of immunological protection against malaria (209, 210).

5.2 Antibodies to merozoites

It has been demonstrated that antibodies against several merozoite antigens function through neutralization (211). Antibodies can inhibit the invasion of red blood cells (RBCs) through binding to merozoite antigens and can inhibit *P. falciparum* growth and multiplication *in vitro* (212). Antibodies can bind to merozoite surface and cause merozoite agglutination, destruction of merozoites by complement-mediated damage, phagocytosis, antibody-dependent cellular cytotoxicity, and antibody-dependent respiratory burst by neutrophils (213). Merozoite surface antigens like *Plasmodium* reticulocyte-binding homologues (*Pf*RH) and erythrocyte-binding antigens (EBA) are also targets of antibody response (214, 215). Anti-*Pf*RH5 antibodies are highly effective at preventing *P. falciparum* merozoites from invading erythrocytes (216). Recombinant monoclonal antibodies against both *Pf*Rh5 and *Pf*CyRPA have been shown to block invasion (217). Interestingly, both non-neutralizing and neutralizing monoclonal antibodies against *Pf*Rh5 can synergize to reduce parasite invasion of RBCs (216). Anti-EBA-175 monoclonal antibodies (R217 and R218) have been described as inhibitory for *P. falciparum* invasion in RBCs (218). Human monoclonal antibodies against various merozoite antigens have been isolated (*Pf*MSP1, *Pf*MSP2, *Pf*MSP3, *Pf*RH5, *Pf*AMA1), and some of these antibodies were seen to exhibit anti-parasitic activity *in vitro* (219). Monoclonal antibodies to MSP1 paralog in *P. vivax* (*Pv*MSP1P) can also reduce parasite invasion (220). Anti-MSP3 antibodies were shown to have anti-malaria activity *via* antibody-dependent cellular suppression of *P. falciparum* (221). The DBP is a vital ligand for *P. vivax* blood-stage merozoite invasion and monoclonal antibodies against DBP inhibited parasite binding to RBCs (222). Human monoclonal antibodies (053054 and 092096) have been shown to neutralize *P. vivax* in *ex vivo* experiments (223). Monoclonal antibodies to *P. vivax* reticulocyte binding protein 2b (*Pv*Rbp2) can inhibit parasite invasion into reticulocytes (224). An antibody against AMA1 exhibits significant inhibitory activity against different *Plasmodium* strains, providing a basis for its therapeutic application (225). Rhoptry (apical organelles involved in erythrocyte invasion) proteins participate in the invasion of red blood cells by merozoites and monoclonal antibodies specific to RAP1 inhibit *P. falciparum* growth *in vitro* (226). A monoclonal antibody (RAM1.25) developed against rhoptry-associated membrane antigen (*Pf*RAMA) exhibited both the growth inhibitory and neutralizing activity against the *Plasmodium* parasite (227).

5.3 Antibodies to iRBCs

The role of antibodies to *Plasmodium* parasite-infected erythrocyte surface antigens (including *Pf*EMP1) in naturally acquired immunity to malaria is still unclear (228). Antibodies targeting VSAs such as *Pf*EMP1, RIFINs and STEVORs proteins expressed during the infected erythrocyte stage are key components of natural immunity to malaria (40) The

antibodies against VSAs work by preventing the parasite's attempts to evade the immune system (229). Antibodies attaching to the surface of the iRBCs can promote phagocytosis and agglutination of iRBCs. Further, antibodies directed against iRBCs can inhibit rosette formation, or schizont outflow and adhesion of the iRBCs to endothelium and epithelium (sequestration) (4). *Pf*EMP1 expressed on the surface of iRBCs is a major target of protective antibodies in malaria (230) and it has been hypothesized that repeated infections are required to elicit a protective repertoire of *Pf*EMP1-specific antibodies (231, 232). Additionally, in pregnancy-associated malaria, antibodies against VAR2CSA (a variant of *Pf*EMP1, which binds to CSA in the placenta) have been linked to protection against malaria (233, 234). The binding of *Pf*EMP1 to CSA receptors allows the sequestration of iRBCs in the placenta and VAR2CSA antibodies function mainly by inhibiting parasite adhesion to RBCs and sequestration along with other effector mechanisms (235). In addition, monoclonal antibodies against *Pf*EMP1 inhibited the formation of rosettes (236). Interestingly, a new class of receptor-based monoclonal antibodies generated by the insertion of a host receptor (collagen-binding inhibitory receptor, LAIR1) into an antibody gene have been shown to agglutinate iRBCs and opsonize them for phagocytosis by monocytes, thereby aiding parasite clearance (237). Monoclonal antibodies to *Plasmodium* schizont egress antigen-1 (*Pf*SEA-1) (expressed in schizont-infected red blood cells), decreased parasite replication by arresting schizont rupture, and maternal antibodies to *Pf*SEA-1 protected infants from severe malaria (238, 239).

5.4 Antibodies to gametocytes

Antibodies against gametocytes can affect the maturation and sequestration of early gametocytes and circulating gametocytes respectively. Additionally, antibodies target gametes that develop in the midgut of mosquitoes (240). Antibodies targeting gametocyte antigens *Pf*s230 and *Pf*s48/45 can show transmission-blocking activity (TBA) by inducing complement-mediated lysis or promoting phagocytosis (240–242). A humanized monoclonal antibody (TB31F) against *Pf*s48/45 which binds to gametocytes and inhibits fertilization. TB31F was capable of completely blocking the transmission of *P. falciparum* parasites from humans to mosquitoes in a phase 1 clinical trial (243). Antibodies to macrogametes and/or zygotes can inhibit parasite development within the mosquito (244, 245). Antibodies to female gamete antigen *Pf*s47 also have TBA and may function by inhibiting ookinete development and fertilization (246). Neutralizing antibodies to *Pf*s25, a zygote antigen, can reduce transmission independently of complement (247). Recently, it has been reported that monoclonal antibodies generated against *Anopheles gambiae* mosquito saliva protein TRIO (AgTRIO) markedly reduced early *Plasmodium* infection in a murine model (248). Human monoclonal antibodies to *Pf*s25, (a gametocyte antigen) can block malaria transmission. Membrane-associated erythrocytic binding protein (MAEBL) is expressed in the liver stages. It is required for sporozoite infection of mosquito salivary glands and antibodies against MAEBL partially inhibit hepatocyte invasion by sporozoites and/or liver-stage development (249, 250). Monoclonal antibodies to *Plasmodium* protein CelTOS strongly inhibited the oocyst development of *P. falciparum* in mosquitoes and neutralized sporozoite hepatocyte infection *in vivo* (251).

Neutralizing monoclonal antibodies raised against the GPI toxin of *P. falciparum* can inhibit the induction of TNF-α. They can also modify the clinical course of infection in animal models of severe disease (252). During *P. yoelii* infection, treatment of mice with anti-IL-10 monoclonal antibodies resulted in substantial prolongation of survival, whereas treatment of mice with anti-IFN-γ monoclonal antibodies exacerbated infection (253). Exported protein 1 (EXP-1) found in the parasitophorous vacuolar membrane seen during the liver and blood stage, contains a defined epitope. This defined epitope is recognized by a parasite inhibitory monoclonal antibody (8E7/55) (254). The GLURP is an exoantigen expressed in all stages of the *P. falciparum* life cycle in humans. It is a target for antibody-dependent monocyte-mediated inhibition of parasite growth, and affinity-purified human IgG antibodies to GLURP can promote a strong ADCI effect *in vitro* (255). A monoclonal antibody directed against EWGWS epitope of Enolase (*Pf*Eno) was found to slow blood-stage malarial parasite growth. It may protect against dual-stage, species and strain-transcending malaria (256). Monoclonal antibodies against pre-erythrocytic stage antigens and erythrocytic stage antigens are currently being explored for therapeutic use. Notably, monoclonal antibodies targeting the sexual stage antigens in mosquitoes can abrogate transmission. Although gametocyte antibodies are largely responsible for reducing malaria transmission, it has been hypothesized that some antibodies can mediate antibody enhancement of malaria transmission (257). While the protective nature of *Plasmodium*-specific antibodies has been demonstrated in multiple studies, few reports have also identified non-protective antibodies (258, 259). Furthermore, the identification of protective antibody epitopes can be useful in developing antibody-guided vaccine designs against malaria. Recently, Murugan et al. identified a conserved core epitope by characterizing 200 human monoclonal *Pf*CSP antibodies induced by sporozoite immunization. This epitope-based approach can be used for rational designing of a next-generation *Pf*CSP vaccine, which can elicit high-affinity antibody responses (260).

6 Host-directed therapies

Host-directed therapy can be implemented during multiple stages of malaria infection by targeting host cell functions which are required for parasite survival and proliferation. Host-

directed therapy does not put selection pressure on *Plasmodium* which prevents the selection of specific genetic variants involved in conferring drug resistance. Therefore, by targeting specific host molecules, the problem of anti-malaria drug resistance can be resolved.

6.1 Host-directed therapy against liver stage

During liver stage infection, the host-directed therapy may prove crucial, as blocking malaria infection during the early liver stage could prevent the progression of sporozoites to merozoites. After invading hepatocytes, *P. vivax* sporozoites transform either into schizonts or hypnozoites. Schizonts are dividing forms while hypnozoites are non-diving or dormant forms. The size of hypnozoites increases slightly with time and are considered to be metabolically active forms (261). Currently, no biomarkers are available to detect hypnozoite infection in humans which makes its early diagnosis challenging. However, one study reported that hypnozoites-infected liver-chimeric humanized mice hepatocytes secrete parasite protein-loaded exosomes in plasma indicating the presence of *P. vivax* infection (262). It has been suggested that the elimination of even a small fraction of hypnozoites could prove to be beneficial for tackling the increasing incidence of relapsing malaria (263). Currently, there are very few approved drugs such as primaquine and tafenoquine which acts against hypnozoites. However, these drugs are associated with complications in glucose-6-phosphate dehydrogenase (G6PD)-deficient individuals (264, 265).

The host factor CD68 is thought to facilitate the traversal of sporozoites through liver-resident KCs making it an attractive target for host-based therapy (18). In one study, it has been reported that monensin, an antibiotic conferred protection against sporozoite infection in a mouse model. Monensin renders host cells resistant to sporozoite infection by inhibiting sporozoite invasion to hepatocytes (266). Recent studies have revealed that a series of host cell endocytic vesicles are sequestered towards intracellular sporozoites which aid in their development during the late liver stage (267). Various host cell proteins, such as COPB2 (coatomer protein complex subunit beta 2), COPG1 (coatomer protein complex subunit gamma 1) or the adaptor protein GGA1 (Golgi associated, gamma adaptin ear containing, ARF-binding protein 1), are involved in trafficking of vesicles toward intracellular parasite (268). Targeting these cellular proteins can impair parasite development in hepatocytes. It has been shown that targeting aquaporin-3 (AQP3), which is a water channel protein contributing to the development of *Plasmodium* during multiple stages of its life cycle, can lead to successful impairment of *P. vivax* liver stage (269, 270). Therefore, the development of AQP3 inhibitors may have an anti-hypnozoite effect which may decrease the prevalence of relapsing malaria. Furthermore, p53, a tumour repressor gene is involved in altering lipid peroxidation in the hepatocytes which negatively impacts the liver stage development (271). Upregulating the levels of p53 leads to a dramatically reduced number of liver-stage parasites (272). *Plasmodium*-infected hepatocytes are thought to be more susceptible to mitochondria-initiated apoptosis. Treatment with a chemical inhibitor which inhibits B-cell lymphoma 2 (Bcl-2) family proteins can result in enhanced apoptosis of infected hepatocytes (272). Another protein of the Bcl-2 family, BCL-xL contribute to *P. falciparum* development in iRBCs. BCL-xL inhibitors impaired parasite growth *in vitro* and induced apoptosis of iRBCs (273). Furthermore, various cellular inhibitor of apoptosis proteins (cIAPs) gets upregulated during *Plasmodium* infection. Liver stage malaria parasite can be controlled by the inactivation of cIAPs which results in TNF-mediated apoptosis of infected hepatocytes (274).

6.2 Host-directed therapy against erythrocytic stage

During blood-stage infection, erythrocyte receptors such as basigin (BSG) and CD55 facilitate merozoite invasion to erythrocytes (275, 276). These erythrocytic receptors could prove to be potential therapeutic targets. Moreover, merozoites can also invade erythrocytes *via* cell surface receptor, ICAM-4 (intercellular adhesion molecule-4) (277). Treatment with ICAM-4 inhibitors could be used to block the entry of merozoites to erythrocytes. Furthermore, several host protein kinases are involved in blood stage development and targeting these kinases *via* kinase inhibitors could prove to be an essential approach against malaria. During blood-stage infection when merozoites invade erythrocytes various protein kinase gets activated (278). Blood stage infection has been shown to activate downstream cell signalling pathways which involve activation of PAK-MEK kinase in host erythrocytes. Although protein kinase inhibitors such as U0126, a MEK1 (MAP/ERK kinase-1) inhibitor, are candidates for host-directed therapy against parasite proliferation in erythrocytes (279), MEK1 inhibitors are associated with cell toxicity. Therefore, further research is required in the development of strategies for reducing toxicity. In erythrocytes, ferrochelatase is an enzyme involved in heme biosynthesis. Ferrochelatase inhibitors have been shown to restrict *Plasmodium* growth inside healthy human erythrocytes *in vitro* (280). Therefore, desferrioxamine, an inhibitor of ferrochelatase could be used in targeted therapy against malaria. Human erythrocytes contain Peroxiredoxin-2 (Prx2), a thiol-dependant peroxidase which protects the erythrocytic cells from the oxidative environment encountered by erythrocytes during malaria infection (281). *Plasmodium* utilizes these peroxidases for haemoglobin digestion which

contributes to its development inside erythrocytes. Treatment with Prx2 inhibitor, Conoidin A renders erythrocytes resistant to *P. falciparum* infection (281). One recent study has shown that selective inhibition of the glycolysis process in iRBCs by Enolase inhibitors (HEX and DeoxySF-2312) could be a novel host-directed therapy against malaria (282).

6.3 Host-directed therapy against cerebral malaria

Aptamers are ss-oligonucleotides (ssDNA or RNA) which can recognize, bind and alter the activity of targeted molecules. It has been suggested that aptamers targeted against host cell-matrix receptors could be used in blocking the interactions between parasites and host cells (283). A combination therapy containing antimalarial drugs and host-directed anti-inflammatory innate defence regulator peptides (IDR-1018) increased the survival rates of malaria-infected mice. Therefore, IDRs along with antimalarial drugs could be a promising adjunctive host-directed therapy against severe malaria (284). Currently, artemisinin is the drug of choice for cerebral malaria and the development of host-directed therapy is underway. In one study, it has been shown that inhaled form of NO (nitric oxide) along with its derivative can be used as an adjunctive treatment against cerebral malaria (285). *PfGPI*-induced host inflammatory responses play an important role in the pathogenesis of severe cerebral malaria. *Pf*GPI stimulates host macrophages and induces TNF-α secretion *via* activating MAPK pathways, including JNK2. Therefore, treatment with JNK2 inhibitors can decrease TNF-α secretion, thereby reducing inflammation in mice models of cerebral malaria (286). Interestingly, treatment of infected mice with NRG1 (neuregulin1), a neuronal growth factor, reduced tissue damage during experimental cerebral malaria (287). The brain microvascular endothelium plays a major role in the pathogenesis of cerebral malaria and molecules, modulators/inhibitors targeting its regulatory pathways are promising candidates in the treatment of cerebral malaria. Repurposing of current therapeutics which modulate the endothelium of the blood-brain barrier such as S1P modulators (neurologic disease) and VEGFR2 tyrosine kinase inhibitors (cancer) could confer neuroprotective activity against cerebral malaria (288).

Furthermore, AQP3 is required by both *P. vivax* and *P. falciparum* for their development during blood stages, therefore, AQP3 inhibitors may contribute to pan anti-malaria activity (289, 290). Although little is known about the role of host-directed therapy for gametocyte stages, host-targeted therapy that reduces gametocyte development and differentiation into male and female gametes could limit their transmission (289, 290). An added advantage of host-directed therapy is that it could be employed against host cellular pathways involved in the production of erythrocytic components that are scavenged by the parasites for their development. These therapies would act by depriving the parasite of these essential components. Since host-directed therapies control host pathways and the parasite has no genetic control over the host proteins, therefore it is less likely that the parasite would develop resistance against these therapeutics (291).

7 Conclusion and future directions

The parasite immune evasion strategies contribute to parasite survival and are considered a big obstacle in developing effective therapeutics against malaria. Research gaps yet remain in our understanding of host-parasite interactions and insights into these mechanisms are of utmost importance for developing effective vaccines and immunotherapies that can overcome immune evasion mechanisms and induce long-lasting immunity against malaria.

RTS,S is the most promising anti-malaria vaccine to date which has completed phase III clinical trials. However, its limited efficacy and geographically regional effect have been seen in many studies. Compared to RTS,S and other subunit vaccines, the whole sporozoites-based vaccine has had more success (292). Despite significant progress in whole sporozoite-based vaccines, the lack of an effective system for *in vitro* production of *P. falciparum* sporozoites warrants more research for malaria vaccine development. Moreover, most of the vaccination studies are based on *P. falciparum* and vaccine research on the second most malaria-causing strain *P. vivax* is lagging far behind. Furthermore, research on *P. vivax* candidates is limited due to difficulties associated with *in vitro* continuous culturing of *P. vivax* and only a few *P. vivax* vaccine candidates have reached to clinical development stages. Therefore, there is an emergent need to expand the repository of *P. vivax* vaccine candidates for demonstrating heterologous protection (293). Although a variety of anti-malaria vaccine candidates have been identified, associated limitations such as poor immunogenicity with limited efficacy impede their success. Additional research is needed for the development of an effective and safe vaccine which can generate long-lasting and strain-transcending immunity in people of all age groups. Using novel immunoinformatics and/or *in silico*-based approaches, a combination of different vaccine antigens from multiple stages of the *Plasmodium* life cycle may prove beneficial for developing a multi-antigen or multi-stage vaccine against malaria. Additionally, improving vaccine protection utilizing a staggered, segmented dosage regimen and other alternative adjuvants along with novel delivery systems must be explored. Development of a variety of vaccine platforms including VLP-based, multi-stage chimeric, GAPs, LARC GAP, mRNA vector-based, CPS-based and nanoparticle-based vaccines along with immunogenic adjuvants that can elicit robust immune responses are currently underway (Figure 1). To improve the affinity and

longevity of vaccine-induced protective antibodies, novel target epitopes should be identified which can induce long-lived protective humoral responses. Vaccine strategies should not only include optimized antibody epitopes, but T-cell epitopes as well for mediating effective Th1 and Th2 responses. More efforts are needed to develop and refine existing animal models for investigating protection mechanisms. Determining immune correlates of protection will accelerate the development of an efficacious malaria vaccine in future.

Since humoral immunity contributes to immune defence mechanisms against malaria, antibody-based therapeutics may prove beneficial in the prevention or treatment of malaria. Monoclonal antibody-based therapy is of particular interest in containment and/or outbreak zones where active malaria transmission is confined to a particular area, season and travellers. Development of human monoclonal antibodies against key vaccine targets of *P. falciparum* and *P. vivax* helps to identify conserved epitopes that aid in vaccine development. Such approaches can also be extended to various stage-specific antigens of *Plasmodium*. Combination therapy with bispecific antibodies or cocktails of antibodies can be prepared that can target different antigens of parasite stages. Various antibody effector functions such as promoting neutrophil burst, NK cell-mediated killing, phagocytosis, agglutination, schizont egress inhibition, rosette inhibition, complement fixation, antibody-dependent complement-mediated lysis, inhibition of adhesion of infected erythrocyte and inhibition of merozoite invasion, have been investigated in various studies (Figure 2). The roles of variation in immunoglobulin allotype, antibody glycosylation and Fc sequence have been less explored. Further studies may help in understanding the immune responses which are necessary for protection from malaria.

Host-directed therapy, which is another major area of therapeutics, has emerged recently. Malaria parasite relies on a network of host pathways which contribute to its development. A wide variety of host factors that are involved in the development of the parasite present novel opportunities for host-directed therapies in malaria. Host-directed therapy particularly targets the host factors which makes parasites deprived of these essential factors needed for parasite invasion, multiplication, and survival inside host cells. Host-directed therapy acts synergistically with anti-malarial drugs and could also be used as novel adjunctive therapy in treating malaria. Furthermore, combining antimalarial drugs with vaccine/s or antibodies and using them as an adjunct therapy show potential to reduce the prevalence and transmission of malaria. It is expected that these combined approaches including antibody-based therapy, host-directed therapy and the development of novel and efficacious vaccines can contribute to the current goal of WHO for a malaria-free world. In summary, a thorough comprehension of the equilibrium existing between the host immune system and parasite immune evasion mechanisms is extremely important for the development of efficient immunological therapeutics.

Author contributions

PC: original draft preparation, reference collection, and manuscript writing; RR: manuscript editing; SK: manuscript editing; SR: conceptualization, supervision, manuscript editing, and proofreading. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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