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Malaria: An Insight into Its Biology, Pathogenesis, Limitation and Opportunities associated with current therapy of Malaria.

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Abstract

The malaria disease is caused by five species of unicellular eukaryotic Plasmodium parasite that are spread by Anopheles mosquito's bite. It remains one of the serious life- threatening infectious disease and causing millions of deaths in 2021. Malaria is controlled with the combination of vector control methods and medications for both treatment as well as prevention. The prevalent utilization of artemisinin-based polytherapy has attributed to significant reductions in malaria deaths, but drug resistance poses threat to overturn this advancement. Understanding the fundamental biological pathways of disease pathogenesis has aided the development of new medicines, insecticides and diagnostics. Several new combination therapy as well as new compounds with effective against medicine resistible plasmodium and their potential to be employed in mono-dose regimens to increase adherence are in clinical trial. This optimistic malaria-eradication programme includes novel strategies that could result in malaria vaccine or vector controlling strategies. However, despite these accomplishments, malaria elimination will require a well-coordinated global initiative on multiple fronts.

1. Introduction

Malaria is a severe vector borne disease. It is caused by *Plasmodium* protozoan parasites and transmits to human when an infected female mosquito of the *Anopheles* species bites them. There are two most common species *Plasmodium falciparum* (P. falciparum) and *Plasmodium vivax* (P. vivax) for which humans are exclusive mammalian hosts. These two species are responsible for largest public health burden(1, 2). According to World Health Organisation (WHO) malaria infected an estimated people causing 4,35,000 deaths in 2017 globally. WHO's latest World malaria report 2021 indicate that malaria infected an estimated 241 million people causing 6,27,000 malaria deaths worldwide in 2020. About 14 million more cases and 69,000 more deaths were reported in 2020 compared to 2019(3).

The clinical symptoms of malaria include fever, shivering, headache, muscle pain, vomiting, severe anemia, spleen enlargement, irreversible coma as well as death if left untreated. The treatment and management of malaria are challenging due to lack of an effective vaccine, ineffective vector control strategies and the growth of drug-resistant parasites. Furthermore, co-infections with malaria and human immunodeficiency virus complicate diagnosis and treatment(4).

The Plasmodium parasite (i.e., dominicana n. sp.) was discovered for the first time in mosquitoes retained in amber (resin) from the palaeogene period, approximately 30 million years ago. In Africa, malaria most probably co-developed with non-human primates. *P. falciparum* may have appeared from gorilla parasite about 10,000 years ago, whereas *P. vivax* have appeared far earlier from non-specific ape hosts. On the opposite side, *P. knowelsi* most probably appeared among rhesus monkeys in Southeast Asia about 4,78,000 -98,000 years ago, although roots to *P. ovale* and *P. malariae* remain unknown, whereas these parasites are

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presently related with gorilla, chimps, and bonobos in Africa(5).

Discovery of Plasmodium Parasites

Malaria was first described in the ancient Chinese medical records in 2700 BC and 1200 years later in the Ebers Papyrus(6). Alphonse Laveran first used optical microscopy to observed that gametocytes from the plasmodium in the blood of the patient in 1878 in Bone city at Algeria(5). Ronald Ross observed that *Plasmodium reticulum* has been spread by culicine mosquitoes in 1897 in India(7). In 1898, Giovanni Battista Grassi, Amico Bignami and Giuseppe Bastianelli had provided evidence that plasmodium parasite could be transmitted from mammal to human via Anopheles mosquitoes(5, 8). During 1947, Henry Shortt and Cyril Garnham illustrated pre-erythrocytic schizonts of *Plasmodium cynomolgi* in the liver of Macaque monkey. Finally in 1982, Krotosky explained malaria relapses by interpreting the dormant exoerythrocytic hypnozoites of *Plasmodium vivax*(7).

Life cycle of Plasmodium Parasite

Plasmodium species are unicellular eukaryotic organisms of the Apicomplexa phylum. Plasmodium species are universal pathogen with complex life cycle that completes in two phases. Sexual phase takes place in mosquito and asexual phase takes place in human host. In humans “blood stage” is particularly responsible for much of the disease pathology. Only 68 species of Anopheles mosquito spread malaria out of the 460 species. *Anopheles gambiae* is a most common malaria vectors which is found in Africa. It lives in areas close by human habitation. It prefers feeding on humans and is long living(9).

The malaria infections starts when sporozoites reside within the mosquito’s salivary gland invade host liver(10). Infected female mosquitoes inject sporozoites with their saliva during a blood meal. Anti-hemostatic and anti-inflammatory enzymes are present in the saliva of mosquito that prevent the blood clotting process and in the pain perception. Each infected bite typically carries 5-200 sporozoites that infect humans. Before reaching the blood stream sporozoites hold on for prolonged time in skin. Only the sporozoites that survive attack of phagocyte can rapidly enter human blood stream through blood vessel, where sporozoites circulate for some time before infecting hepatocytes(11).

As per figure 1 life cycle of plasmodium parasite completes in following sequence.

(A) Malaria infection begins with the injection of sporozoite into the middle layer of the skin by female mosquito.

(B) The sporozoites enter the blood vessel and they are carried to the liver, where sporozoites exit sinusoids (blood vessels of hepatic microcirculation) by means of stellate macrophages or endothelium as well as invade liver cell. Active invasion of liver is preceded by disruption of plasma membrane (cellular traversal) untill a suitable liver cell is found. During active invasion of hepatic cell sporozoite uses part of the host cell membrane to form a Parasitophorous Vacuole Membrane (PVM). Sporozoites undergo asexual reproduction until ten thousands of daughter merozoites are discharged into the blood vessel in packets of merosomes.

(C) In blood, sporozoites come across erythrocytes and start a persistent cycle of asexual schizogony.

(D) A fraction of asexually reproducing merozoites is modified to undergo gametocytogenesis.

(E) Gametocytes sequester and mature within the bone marrow before entering the peripheral circulation. Mosquito vector takes up the gametocytes concentrated in skin capillaries in another blood meal. After three rounds of mitosis, each male gametocyte in the mosquito’s gut produces eight microgametes . Female gametocyte produces macrogamete.

(F) Male microgametes flagellated motile forms that seek out female macrogamete. Zygote is formed by fusion of micro and macro-gamete. Over the period of 24 hours, a zygote evolves into ookinete. Ookinete is motite form that moves through the

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mosquito's midgut epithelium, where ookinete develop into encyst then become an oocyst. Oocysts grow, rupture and release sporozoites, which migrtae from mosquito's abdomen to the salivary gland(12).

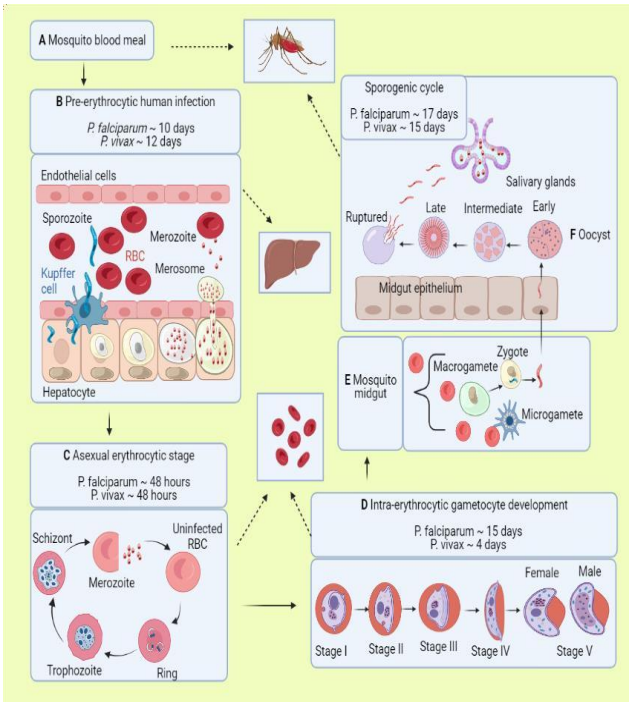

Figure 1. Life Cycle of Plasmodium Parasite

2. Infection of Liver

After being bitten by an infective mosquito, sporozoites start leaving the bite site and enter the blood stream by passing through a skin capillary, where they are transported to the network of hepatic capillary (sinusoids). The sporozoite then passes through endothelial barrier and into the liver parenchyma. Here, sporozoites pass through multiple liver cells before undergoing functional changes that allow them to invade and colonise hepatocytes. The sporozoite excretes the content of micronemes and rhoptries during and after invasion. Micronemes and rhoptries are unique set of invasive or secretory organelle in which the integral proteins facilitate molecular interaction with the human cell. The underlying molecular mechanism and order of occurrences in the cell intrusion have been thoroughly studied with respect to merozoite intrusion of erythrocyte, but significantly less is understood about sporozoite infection of liver cells. Merozoites and sporozoites cell invasion share similarities including parasite's actin-myosin motor, which enables active entrance into host cell and invagination of human cell's plasma membrane throughout cell invasion that safely establish the subcellular protozoa within the host cell membrane, recognised as the parasitophorous vacuole membrane (PVM). PVM is widely adapted because of growing hepatic stage parasite and acts as a barrier as well as a channel for communication and nutrient acquisition.

As per figure 2 sporozoites invade host hepatocyte in following sequence.

(A) The flagellated sporozoite's surface is encased with circumsporozoite protein (CSP), that captures a glycoprotein on the hepatocyte surface called heparin sulfate proteoglycnas (HSPGs). The sporozoite's apical end contains organelles such as rhoptries (Rh, pink), micromenes (Mc, orange), and possibly dense granules (DG, blue). The contents of these organelles are crucial for hepoatocyte invasion.

(B, C) Contact of sporozoite with invasion permissive hepatocytes triggers circumsporozoite protein (CSP) processing, which leads to the loberation of invasion essential proteins out from the micronemes via an

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unknown mechanism(13). Thrombospondin-related adhesive protein (TRAP) is one of the protein that is essential for invasion. The cytoplasnic domain of TRAP binds with HSPGs on the liver cell surface as well as the sporozoite internal glidesome complex to provide traction for the intruding sporozoite at moving junction(shown as grey ring). P52 and P36 are two micronemes proteins that also play role in the invasion process. They may interact with one other in addition to the hepatocellualr Ephrin A2 receptor (EphA2). The P52/P36/EphA2 axis emerges to be important for parasitophorous vacuole (PV) formation. The hepatocellular receptor CD81 is also vital for invasion process and formation of PV, but it is unclear whether sporozoite interact with it directly. The internal glideosome complex is anchored by inner membrane complex (IMC, shown in yellow), allowing sporozoites to move into hepatocyte. Hepatocellular invasion takes place via the junction in motion at entrance point as well as it is followed by polycondesation of F-actin in collaboration with Arp2/3. Hepatocellular invasion causes the hepatocellular lipid bilayer to infold and discharge of rhoptry proteins such as ROP2, ROP4 and RAP2/3.

(D) As a result of successful invasion sporozoites staying inside a membrane (PV) enclosed with parasiophorous vacuole membrane (PVM) of hepatic origin. The PVM is significantly altered by two protein UIS3 and UIS4. UIS3 (up-regulated in infective sporozoite 3) or UIS4 (up-regulated in infective sporozoites 4) is parasite protein that releases and transported to PVM, as is Exp1 (Exported protein 1). During dedifferentiation of sporozoite, an IMC (dashed yellow line) as well as apical organelles are degraded.

(E) The nascent hepatic stage trophozoite stays inside a PV posseses cellular membrane still encased by CSP, and is enclosed by PVM (blue)(14).

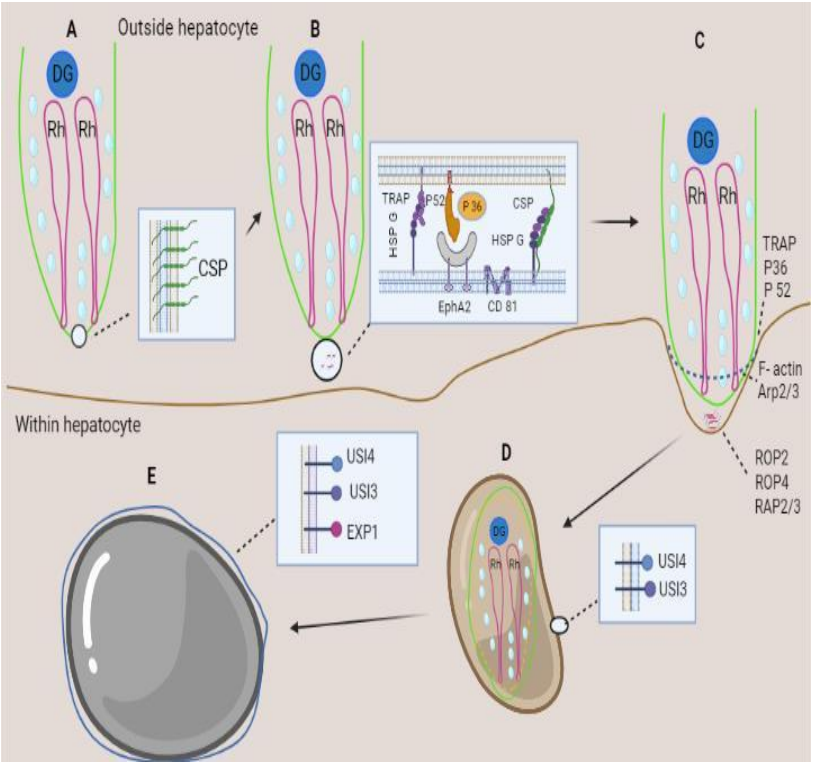

Figure 2. Sporozoite Invasion of Host Hepatocytes

As previously stated, the beginning of hepatic phase development is complicated process that begins when sporozoite recognises a suiatble hepatocytes for infection. After invasion of suiatble hepatocytes, liver stage development of parasite takes place. The figure 3 describes mechanism and sequence of event in liver stage development.

(A) The tubulovesicular network (TVN) (shown in blue) is lipid bilayer bound

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extension and whorls that produced by parasitophorous vacuole membrane (PVM). The TVN interacts with hepatocellular autophagosomes (that express Atg8/LC3) probably for uptake of nutrients. IBIS1, EXP1, UIS4 and UIS3 are the plasmodium proteins expressed on the PVM/TVN. Parasite protein has been shown to interact host liver fatty axis binding protein (L-FABP). Protein B9 is associated with the parasite plasma membrane protein (PPM) and renowned toward being vital for hepatic stage development.

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(B) As parasite matures in the hepatic stage, numerous PPM invaginations takes place (cytomere formation), which is followed by the MSP1 as well as ZIPCO expression. Furthermore, LISP1 as well as LISP2 expression takes place upon PVM

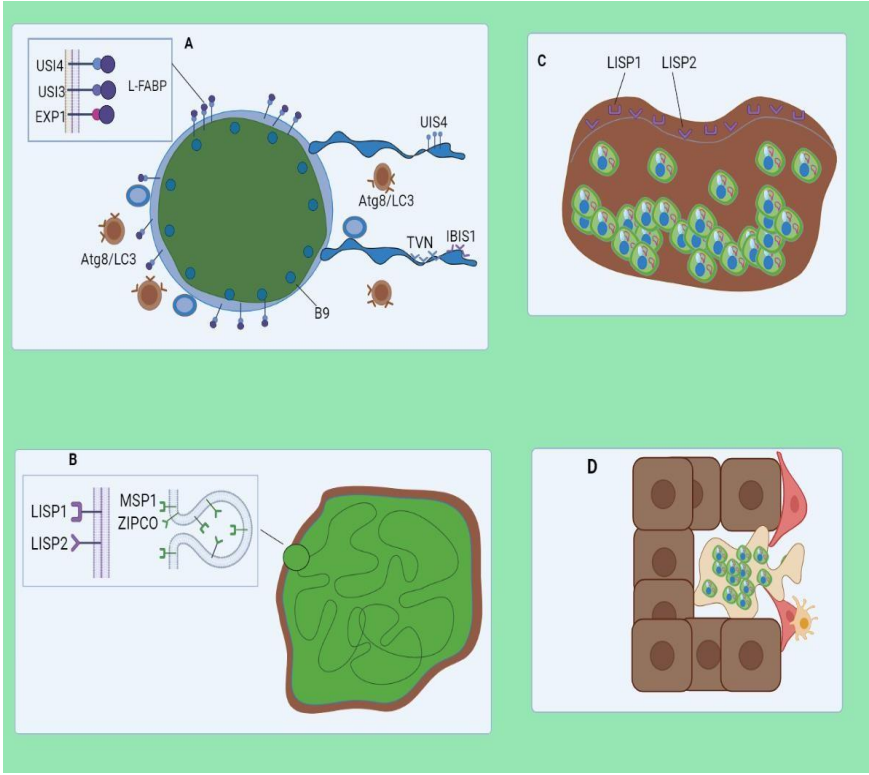

Figure 3. Liver stage development

(C) At the end of hepatic stage development, independent exoerythrocytic merozoites start to form, PVM erodes (a process requiring LISP1), and LISP2 is liberated into the liver cell of the host.

(D) Merosomes and merozoites are enclosed by hepatocellular membrane and they are discharged into bloodstream through hepatic sinusoidal capillary that is defined by epithelial cells and hepatic resident macrophages, as well as the Kupffers cell (yellow)(15, 16).

There are some more proteins that are involved in liver infection of parasite apart from the proteins discussed above. As mutant sporozoite cannot enter the circulation from dermis due to lack of function, they exhibit normal gliding motility. Trap like protein (TLP) plays role to exit sporozoite from dermis(17). Proteins that disrupt plasma membrane called traversal protein and include SPECT (sporozoite micronemes protein essential for traversal), SPECT2 (also known as perforin like protein 1, PLP1), CelTOS (cell traversal protein for ookinetes and sporozoites), phospholipase and gamete egress and sporozoite traversal protein

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(GEST)(18, 19, 20). Tetraspanin CD81 or scavenger receptor B1 (SR-B1) is membrane proteins of human hepatocellular essential for *Plasmodium falciparum* sporozoite to form parasitophorous vacuole (PV)(21). Once hepatocellular infection has been established, a sporozoite evolves within next 2-10 days into a hepatic stage (HS) or exo-erythrocytic form (EEF), as well as development come to end with discharge of as many as forty thousand merozoites for each liver cell into bloodstream by sprouting of parasite filled vesicle known as merosomes(22).

3. Invasion of Erythrocyte

Once merozoites released into hepatocellular circulation, free merozoites infect red blood cells in quick, energetic and multistep process involving pre-invasion, active invasion as well as echinocytosis, which are accomplished within 2 min(23). During pre-invasion, initial interaction and deformation of erythrocyte membrane occurs. Active invasion involves apical interaction. Echinocytosis is rapid shrinkage of red blood cells with evenly spaced projections and followed by recovery of invaded host cells(24).

Low affinity contacts are created with erythrocyte cell membrane, during pre-invasion. Reconfiguration of merozoite is essential to facilitate nearer interaction between parasite ligand and host cell receptor. Tight junction formation takes place after reorientation. Pre-invasion involves two proteins erythrocyte binding-like proteins (EBLs) as well as *P. falciparum* reticulocyte-binding protein homologs (*Pf*Rhs). Such protein ligands interact with specific receptor such as glycophorin A, B, C as well as complement receptor 1 (CR1). In *Plasmodium falciparum*, complement decay accelerating factor upon host erythrocyte is necessary for the invasion of all *Plasmodium falciparum* strain(25). Merozoite surface protein 1 (MSP1) is a major glycosylphosphatidylinositol anchored protein(26). There are nine GPI anchored proteins and all are potential erythrocyte ligand. Some of GPI anchored protein (e.g., Pf38) have a more apical localization consistent with different role in invasion(27).

In all strains, an aggregation of *Plasmodium falciparum* protein reticulocyte-binding protein homologue 5 (PfRH5), PfRH5 interacting protein (PfRipr) as well as cysteine rich protective antigen (PfCyRPA) with extracellular matrix on the erythrocyte surface is also necessary for invasion(28, 29). PfRH5 has been investigated as a possible vaccine contender, as well as antibodies to extracellular matrix have really been examined as a possible therapeutic strategy(30, 31). During the binding step of PfRH5-PfRipr-PfCyRPA-basigin, a pore forms between merozoite and erythrocyte, and this triggers calcium (Ca^{2+}) release and allows insertion of merozoite released protein into the erythrocyte lipid bilayer. Such proteins are released from the tiny secretory organelles that aggregate at the anterior surface of the merozoite, these secretory organelles are known as micronemes. Rhoptry neck protein 2 (PfRON2) is released from the rhoptries' necks. The interaction between PfRON2 as well as apical membrane antigen 1 (PfAMA1) on merozoite's surface is necessary to facilitate tight junction formation prior to internalisation process(32). The PfAMA1 is also studied as a vaccine candidate(33).

After active invasion, membrane fusion takes place at the merozoite's posterior end to encapsulate the parasite inside the parasitophorous vacuole and red blood cell. Echinocytosis then takes place, causing the red blood cell to shrink as well as produce an edgy protrusion. This could be due to Ca^{2+} influx into the red blood cell during interaction of the PfRh5 complex with basigin.

Once parasites gain entrance into the erythrocyte, they release large numbers of proteins into the cytosol and membrane of host cell that regulate the nutrient acquisition, cell adhesions and sequestration in tissue, and pathogenesis(34, 35). In the erythrocyte, replication of parasite needs sustained pool of nucleic acid bases for the biosynthesis of DNA and RNA, which can be inhibited by many antimalarial drugs. Daraprim, P218 and cycloguanil inhibit *Plasmodium falciparum* dihydrofolate reductase (PfDHFR). The Atovaquone inhibits the transcription of chondriosomal gene *pfcytb* (which encode *Plasmodium falciparum* cytochrome b) and prevents the formation of oxidised coenzyme Q.

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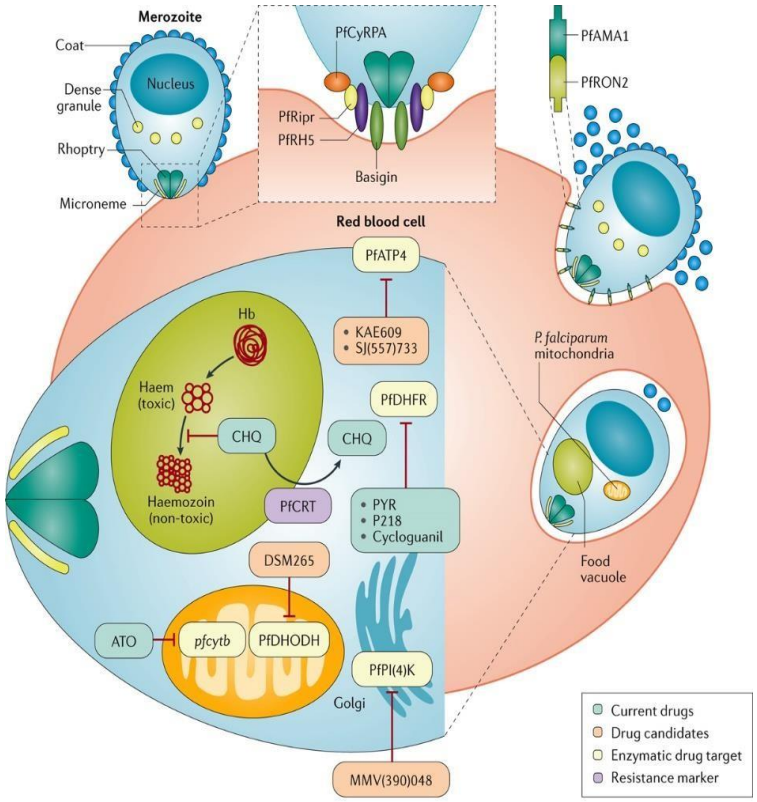

Figure 4. Invasion of Erythrocyte and Replication

Plasmpodium parasites are auxotropic for all amino acids they required (that is, they must obtained all of theses from food because they can not synthesize them from precursors). All amino acids are provided by haemoglobin digestion in a specialised food vacuole, with the exception of isoleucin that should be acquired from other host cellular components(39). Digestion of haemoglobin additionally liberates heam, which is poisonous to parasite and, consequently, is polymerized into haemozoin. Haemozoin is a blue-colored malaria pigment and it is an insoluble crystal which entraps the toxic metabolite(40). It remains unclear that how haem polymerization is faciliated by the parasite. Within the food vacuole, a complex of numerous parasite proteases as well as haem detoxification protein (HDP) has been discovered. *In vitro* experiments have exhibited that elements of this complex (such as falcipin 2, HDP and lipids) can catalyse formation of haem into haemozoin(41). Chloroquine acts by inhibiting heam polymeriation in food vacuole and can be thrown out from food vacuole by the *Plasmodium falciparum* chloroquine resistance transporter (PfCRT). Plasmodium expresses an vital cellular membrane Na^+ export pump [*Plasmodium falciparum* p-type ATPase 4 (PfATP4)], which can preserve Na^+ homeostasis during nutrition acquisition(42, 43, 44). KAE609 is phase II clinical contender and SJ (557)733 is preclinical candidate, both inhibit PfATP4(44, 45, 46). *Plasmodium falciparum* phosphatidylinositol 4-kinase (PfPI(4)K) is needed for the formation of transport vesicles, which are necessary to induce membrane alterations during membrane ingression in the late achizont stage. The clinical candidate MMV(390)048 is in phase I and inhibits (PfPI(4)K)(47, 48).

Transition to Transmission

During cycles of schizogony in the blood stream, a small proportion of parasite undergo development change, committing to sesxual development and producing male and female gametocytes. Malaria transmission is dependent upon the development of

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sexual stage, which has been recognised as possible intervention point via transmission blocking drug or vacccine. Although molecular events surrounding this development change are unknown, the timinig of transition take places at some point during the previous schizogony cycle and daughter merozoites from separate schizont infected cells are commited to grow into either gametocytes or asexual schizont. Environmental stimuli including high parasitemia and chloroquine exposure are related with incresed conversion to gametocyte production, specifying that plasmodium perceive their surrounding. Extracellular vesicles containing protein DNA and RNA allow for cell to cell communication, which increases gametocyte production (49, 50).

Epigenetic governance is important for controlling sexual differentiation. AP2-G is transcription factor and a key regulator of gametocytogenesis(51). During gametocyte maturation, gametocytes remain sequestered inside bone marrow, avoiding splenic clearance until they appear into peripheral blood circulation for an undefined period of time before being reabsorbed by feeding mosquitoe(52).

Human cell remodelling as well as immune Evasion

Following invasion, the parasites initiate a renovation process that transforms a ultimately differentiated cell devoid of most organelles into one that allows intracellular plasmodium to grow as well as hide from host defence. It accomplishes this by exporting large numbers of protein from the plasmodium as well as beyond the PVM to variuos site within the plasmodium infected erythrocyte.

Apart from remodelling of host cell, malaria parasite is encountered by human immune system. Malaria parasite first enters in the immune system of human, once sporozoites are introduced into the skin, where sporozoites might undergo phagocytosis by dendritic cell for innate immunity in the lymph node(53).

Although the diameter of mosquito's proximal duct limits entry of number of sporozoites that really can enter it, the transmission possibilities are great whenever the human has been bitten by mosquitoes, which transmit a large number of sporozoites (54). Sporozoites have ability to supress the fuction of Kupffer cell (recognised as stellate macrophages) and repress gene expression that encodes MHC class I molecules. These are important for immune evasion in liver and can be supressed by sporozoites (55).

Following invasion, large number of proteins are exported into the host cell by parasite. These proteins leave endoplasmic reticulum (ER) through a use of vesicle-mediated secretory pathway, cross the parasite membrane (PM), and enter the parasitophorous vacuole (PV). Protein translocon (PTEX) is present at the parasitophorous vacuole membrane (PVM). PTEX mediates export of protein into the host cell. Flow of membrane material inside the human cell is regulated by plasmodium derived vesicular structures including EDV (electron dense vesicle) and J-dots as well as membranous organelles. Their final destination is the cytosol of erythrocyte, cytoskeleton, or membrane. Knob-associated histidine rich protein (KAHRP) causess elevated points at the red blood cell membrane (known as knob). The major virulence factor *Plasmodium falciparum* erythrocyte membrane protein 1 (PfEMP1) is expressed in the extracellular environment from knob present on eryhtrocyte. PfEMP1 was responsible for cytoahesion to the human cell surface receptors. Exported plasmodium proteins significantly remodel the cytoskeleton of erythrocyte to withstand shear stress and extreme heat. New permeability pathways (NPP/PSAC) have been formed at the red blood cell membrane permitting the plasmodium to harvest nutrients and remove waste. Toxic heme is collected and deposited inside the plasmodium's food vacuole(56, 57).

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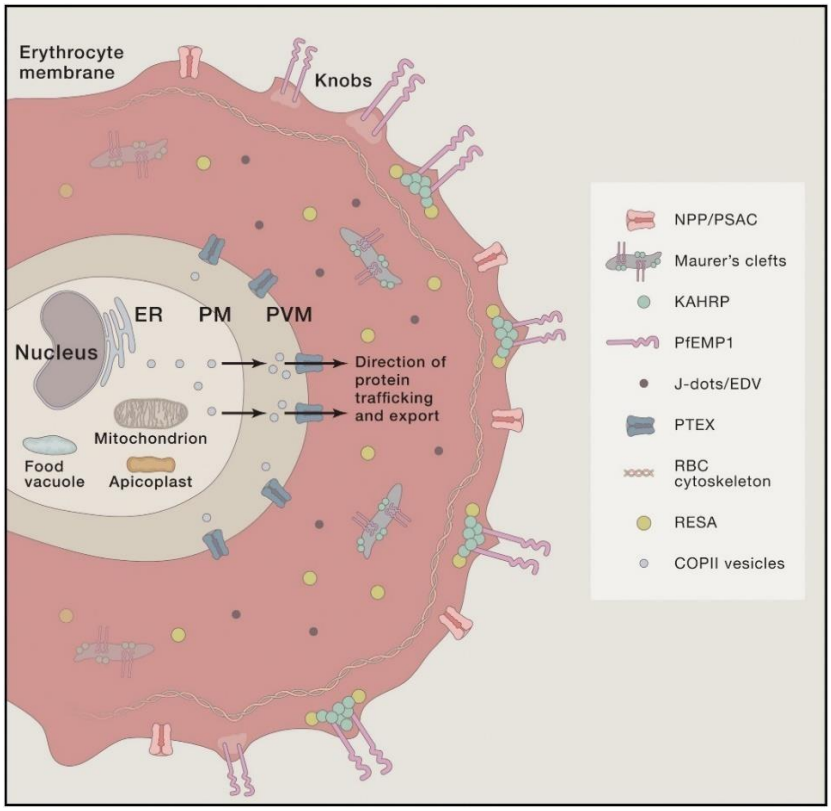

Figure 5 Protein export induced modifications in the host erythrocyte

Naturally Acquired Immune response to Malaria

Naturally acquired immunity refers to an individual's ability to build an adaptive immunity towards plasmodium infection and sickness with time and exposure that defends them against the harmful effects of the parasites(58).

Severity of disease with respect to parasite burden as well as the potential for complication is determined by the extents of the protective immunity attained by the host organism(59). Protective immunity can aid in reducing the intensity of it's manifestations and the possibility of developnig severe disease(60). Immune protection is thought to be induced by circulating IgG antibodies that bind to surface protein on sporozoites (there by preventing hepatocellular invasion) and merozoites (there by blocking blood cell invasion). Adults develop partially protective immunity in greater transmission regions where malaria is prevalent all year. Young infants (less than six months) are also protected, most likely due to antibodies passed down from their mother, so although children aged six months to five years possess the least degree of innate immunity and are particularly vulnerable to developing severe parasitaemia wth risks of complications and death. Individuals in low transmission or weather dependent malaria regions build reduce degree of innate immune protection and usually have worse symptomatic malaria when infected. This relationship between innate immunity and malaria severity presents a challenge for effective malaria treatment programmes; as disease and it's transmission rates declines, rising numbers of individuals will end up losing their immunological defence as well as become vulnerable to serious disease. The emergence of disease again in regions that had been disease free for several years could be fatal in the short to medium term and, thus, well-organized surveillance is needed(13, 61).

Pathogenesis and Clinical Features of Disease

Malaria infection invariably produces a illness that is accompained by fever in a naive individual. The accompanying symptoms are vauge frequently include headache, and muscle pain. Even with significant exhaustion, if the symptoms are cured

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with appropriate medications at this stage, they will go away in few days. As in instance of *Plasmodium falciparum*, absolute tratment will abolish the infestation as well as any reocurrence of symptoms indicates insufficient treatment, drugs resistance as well as fresh infection. As in particular instance of *Plasmodium vivax* and *Plasmodium ovale*, subsequent infestations may reappear at intervals due to reactivation of the dormant hepatic-resident hypnozoite stage, except if this is removed by long treatment with an 8-aminoquinoline. Usually, following a period of changing severity of symptoms, illness stabilises and parasite abundances are restricted to a low level, with manifestations reappearing at intervals over the following weeks and months, related with rises in parasitemia. Typically, successive waves of parasitemia are usually with fewer and milder symptoms until infection eventually resolves(62).

Severe Malaria

In a fraction of untreated or inadequately treated patients the initial infestation does not resolve and evolves to severe or complex malaria, which may be fatal. The severity of disease varies significantly with age as well as transmission level. The majority of malaria deaths in Africa occur in a child and are caused by three syndrome that can appear separately or in a combination. These are Cerebral malaria (CM), Severe anemia and Respiratory distress(63).

CM is deifined as the presence of *Plasmodium falciparum*-induced coma. Seizures, retinopathy and brainstem alterations as a result of high intracranial pressure as well as brain swelling are also diagnostic characteristics observed during cerebral malaria(64).

Respiratory distress with severe malaria in children is accompanied by metabolic acidosis, which is primarily caused by oxygen starvation in tissue. Although accute plasmodium infection is related with destruction of infected as well as uninfected erythrocyte that is a caused of the severe malaria anemia in young children.

Due to naturally acquired immunity, serious malaria in adolescent and adults is extremely rare in of Africa's stably endemic regions. As a result, our comprehension of the disease in older people is based on research from low transmission areas around the world, especially Asia. Severe malaria is a multi-system ailment frequently causes significant renal and liver dysfunction (unusual in child). Respiratory distress is frequently caused by edema of lungs, which occurs infrequently in child but has a high death rates in elders. As earlier in extremely endemic regions of globe experience decrease in transmission (and thus population immunity), the average age of serious disease and death is increasing, as well as the severe disease trend shifts to that observed in older people around a globe(65).

Pathogenesis of Severe *P. Falciparum* Malaria

The primary features of *Plasmodium falciparum* infestation are massive plasmodium growth, initiation of inflammatory pathways in human as well as microvascular obstruction because of sticking of fully developed plasmodium to blood vessel and endothelial activation. These features play role in the pathogenesis of serious disease and result in death(66). Exponential plasmodium growth of more than tenfold on alternate day indicates, elevated overall body parasitemia is ahieved quickly. The significance of this may be obscured by the phenomenon of plasmodium biomass sequestration sticking with the lining of blood vessels, implying that plasmodium mass estimated by microscopy is underestimated. Histidine-rich protein (HRP), for exapmle, provides estimates of plasmodium biomass that are much more understandable(67).

The proliferative stage of intravascular plasmodium growth triggers an accute inflammatory response. Studies have proposed that intense malaria develops as a result of aggressive or poorly regulated responses that have evolved primarily to regulate acute infection(68). Systemic levels of several pro-inflammatory cytokines are associated with increasing severity and death in a number of studies. Birth IL-1β levels have been shown to be predictive of future IL-1β levels as well as risk of severe malaria in adolescence. A strong pro-inflammtory respose may contributes to the progression of severe disease in a number of ways such as systemic as well as local effects(68, 69).

Microvascular Obstruction

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Microvascular refers to small blood vessels with a diameter of less than 100 micrometres. A key feature of severe malaria caused by *Plasmodium falciparum* is impairement of epithelial blood flow related with immobilization of mature plasmodium parasites in microvascular beds. Both end organ failure, particularly in the brain, as well as systemic effects like metabolic acidosis are caused by this. Rectal mucosa and retina are two tissue beds where adsorption and susequent impaired blood circulation have been directly identified and quantified. These tissue beds also exhibit strong correlation in terms of clinical outcome, histologically features of subjects who pass away from disease, and other prognostic marker such as acidosis(70).

Sequestration is mediated by the implantation and display of modified proteins that can bind to receptor on a variety of different cells, which modifies the erythrocyte surface. This seems to include a significant amount of receptor on endothelial cells (which leads to attachment within vessels) as well as binding to uninfected red blood cells (a phenomenon called rosetting) or to activated thrombocytes, which results in development of clsters of infected cells. Both clumping and rosetting are associated with disease severity and likely contribute to microvacular obstruction(71).

Endothelial Activation

Endothelial activation plays a crucial role in the microvascular pathogenesis of *Plasmodium falciparum.* As nitric oxide bioavailability reduces, the levels of angiopoitein 2 rise, as well as these markers are correlated with outcome(72, 73). Endothelial activation boosts the expression of several receptor on which the infected red blood cells can attach. Although the systemic effects from several cell signaling molecules may be significant in causing endothelial alterations, the interaction of infected erythrocyte with the endothelium may cause local endothelial activation and resulting in vicious circle. Recently, the discovery of endothelial protein C receptor (EPCR) to be a ligand for PfEMP1-mediated infected eryhrocyte binding offers a possible clarification for how cytoadherence may casue local endothelial malfunction and how the brain may be especially vulnerable to risks of sequestration. EPCR is found in the majority of vascular beds and cotributes to endothelial stabilization by stimulating protein C activation. Infected erythrocyte binding to EPCR via CIDR domain of PFEMP1 inhibits protein C activation and results in highly localized coagulopathy(74). It is hypothesized that even though EPCR-mediated cytoadherence will occur in numerous vascular beds, the localized coagulopathy is more likely to happen within the brain because of low fundamental expression of EPCR or TM. The fact that the PfEMP1 variants that can bind to EPCR have been linked to severe malaria suggests a possibly essential unifying association between parasite and human host factors mediated severity(74, 75).

Interaction with Other Infections

Malaria is a particular problem in susceptible communities where multiple and ongoing health risks frequetly interact. In both children and adults, AIDS is a serious risk factor for severe illness and mortality due to malaria. Malaria in pregnant women is linked to higher risks of AIDS infection transmission to the fetus(76). From a public health standpoint, the most significant interactions are still with invasive bacterial infections. The prevalence of concurrent infective bacterial illness in African children suffering from severe malaria is far greater than what could happen by chance(77). Most importantly, case mortality rate is much greater in individulas those with dual infections. Several mechanism for asssociation have been offered, including gram-negative pathogen translocation through leaky bowel, particular macrophage malfunction, and functional hyposplenism(78). Malaria is estimated to be accounted for fifty percentage of all incidents of inflammatory bowel disease (IBD) in some areas, leading to counterintutive assumption that it may kill more people indirectly than directly. The reality that huge declines in all cause peadiatric mortality have occurred in a very short period of time while aggressive malaria control has strongly supported this idea(79).

Malaria in Pregnancy

Malaria during pregnancy has been linked to pregnancy loss as well as serious malaria in the woman with a particularly high possibilities of hypoglycemia in non-immune populations. Malaria infection casuses maternal anemia or low birth

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weight in disease endemic regions, where populations develop immunity to some extent(80). Even though sometimes this is referred as loss of immune protection, it is not strictly correct; instead the appearance of the fetal organ provides a fresh location for plasmodium sequestratation via the selection of plasmodium capable of binding to chondroitin-sulfate A (CSA) mostly on the syncytiotrophoblast(81). The enhanced frequency and mass of peripheral pathogens observed during pregnancy are the result of spillover out of a latest "privileged" location within individual who still has functional immune responses. During subsequent pregnancies, women develop antibodies against specific PfEMP1variants engaged in CSA-mediated cytoadherence or female in subsequent childbirths are significantly protected towards negative effects of infection(82).

Challenges in Antimalarial Therapy

Chemotherapy is currently the only approach for treating and controlling plasmodium infection. Recent chemotherapeutic drugs for trearment of disease comprises several problems relating to efficacy, saftety as well as emergence of drug-resistance parasites because of complex life cycle as well as biological pattern of the plasmodium parasite(83).

Toxicity of the antimalarial drugs

Patient compliance is the most significant factor in drug use or its efficacy. The drug's toxicity must be balanced balanced against it's efficacy and risk of disease, in other words drug must cause less negative impact than the disease itself. When treating malaria, the doses administered to the individuals should be considered. Many antimalarial drugs are being evaluated for tolerability, but assessing adverse effect, undesirable effect, adverse incident, unwanted outcomes as well as medication-related toxicity is challenging due to the lack of good excellent tools to evaluate the side effects(84).

The most appealing natuarlly occurring sesquiterpene lactone drug as well as its derivatives such as artemether, arteether, and sodium salt of artesunate showed no severe adverse effects. Moreover, inadequate clinical studies to determine the toxicity prevented scientists from stating artemisinin to be compeletly safe. However, they are extremely safe and effective. Current experimental and clinical research indicates that prolonged accessibility of artemisinins may lead to toxicity (rarely produce neurotoxicity as well as allergic reactions). In comparison to treatment via intramuscular injection, the short-term peak concentrations of artemisinins after oral ingestion are followed by rapid clearance. In contrast to human studies, the majority of animal experiments clearly revealed significant toxicities.

Even at higher doses, chloroquine is considered a safe drug, but it does cause minor side effects including reversible implication regarding optical accommodation that can possibly affect eyesight. It also has an irreversible binding to melanin. As a result, person suffering from rheumatoid arthritis who are treated with administration of high dose of chloroquine for prolonged period encounter chloroquine accumulation in retinal melanin. According to some reports, chloroquine can aggravate psoriasis in patients who are light intolerant(85). Chlorguanide is however thought to be non-toxic at daily dose of 200 mg. Moreover, at doses over 200 mg, there have been reports of reversible alopecia as well as aphthous ulceration (mouth ulcer), nausea, and gastric irritation(86). These adverse effects are also frequent with other antiplasmodial medications. The combination of chloroquine and proguanil is well tolerated. Moreover, gastrointestinal upset and ulcers of mouth continue to be reported. Sulfadoxine/pyrimethamine is similarly well accepted, but it is currently not used due to it's association with Stevens-Johnson syndrome as well as toxic necrolysis. Mefloquine is one more effective antimalarial medication. Despite the fact that the most patients tolerate it well, dose-related severe neuropsychiatric toxicity may occur. Quinine rarely causes cardiovascular and CNS toxicity, but it can cause hypoglycemia. Furthermore, halofantrine is unsuitable for prevalent use because of it's potential for cardiotoxicity. Mepacrine, sulfonamides, dapsone as well as amodiaquine have also been withdrawn from the use due to frequent occrrence of adverse side effects(87).

Nanotechnological strategies for malaria therapeutics

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Recent anti-malarial therapy primarily based on chemotherapy, which causes serious side effects and growth of parasites resistant to drugs, ultimately leading to failure of treatment. To address the therapeutic failures of anti-malarial medications, new drug development is urgently required; however, the drug discovery as well as development process is costly and time consuming(88). Many academies, research institution, pharmaceutical companies and non-profit organizations such as Medicines for Malaria Venture (MMV) around the world have designed, synthesized novel anti-plasmodial compounds and evaluated them against malaria parasite in *in vitro* as well as *in vivo* models. However, preclinical studies have revealed some level of toxicity(89). The complexity of plasmodium life cycle, the dispersion of erythrocytic as well as exo-erythrocytic stages of plasmodium parasite in the host, and lack of knowlwdge about biology and pathology of plasmodium parasite have made it difficult for researcher scientists to discover new malaria medications(89, 90).

Lately, nanotechnological strategies have earned prominence for target specific delivery of medications with better safety and efficacy(91). The nanomedicine is a branch of science with broad application and has a significant effect upon biotech as well as pharmaceutical firms in designing and developing nanostructured (1-1000 nm) materials encapsulated with antibiotics as a suitable approach to overcome the complication associated related to current antimicrobials(92, 93). A primary objective of the nanotechnology based malaria therapy is to target parasite infected red blood cells and intracellular parasite vacuoles with anti-malarial agent encapsulated in nanomaterial. Furthermore, use of nanomaterials allow for increased efficacy, safety, selectivity, changes drug degradation, and supports continuous drug release directly at the target sites. Encapsulating multiple chemotherapeutic using nano-vectors enables combinatorial treatment, which may have a synergistic effect(94). Numerous nanoparticle such as liposomes, solid lipid nanoparticles (SLNs), nanostructured lipid carriers (NLCs), metallic nanoparticles, nano-emulsion, and polymeric nanoparticle have been investigated and found to be very adavantageous as nanocarriers for drug delivery applications(95, 96). These nanotech-oriented formulations may testify to excced the limitations of current therapies in terms of safety, efficacy as well as cost effectiveness, thereby improving patient adherence to treatment(97). Liposomal amphotericin B was recently approved by FDA for intervention of visceral leishmaniasis, a neglectes tropical protozoal parasitic disease with improved efficacy and safety(98). Surface modification of anti-malarial nanoparticles enhances target selectivity and specificity, resulting in enhanced safety or efficacy. Numerous research teams have invented nano-based dosage form with specific anti-plasmodial drugs and tetsed them on parasite-infected animal models, with promising results; however, greater research community attention is required to combat such poverty-stricken tropical infectious diseases(99).

Antimalarial Drug Resistance

Emergence of resistance to medications is another major challenge in anti-malarial therapy apart from safety, efficacy and toxicity.

Antimicrobial Resistance (AMR) occurs when microorganism such as bacteria, viruses, fungi and parasite develop the ability to adapt and develop in the presence of medication that previously harmed them(100).

Eemergence of antimalarial resistance has enhanced the death and morbidity rate that have been reduced thus far by malaria control program. Monitoring resistance to available antiplasmodial drug aids in the execution of effecient drug guidelines, through the use of *in vivo* efficacy test, *in vitro* drug vulnerability studies or determination of molecular markers. Understanding the mode of action of the antimalrial drugs is critical because it is a major contributors to drug resistance emergence and spread. Antimalarial drugs has been used in combination therapy rather than monotherapy to increase drug efficacy and postpone emergence of resistant parasite. Ever since, ACTs have been globally and successfully used to treat malaria. Artemisinin resistance has recently been observed throughout South-eastern Asia, raising the international concern about malaria treatment as well as control.

The emergence of antiplasmodial medication-resistant parasite will merely hinder the malaria

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control efforts and it will also necessitate development of new treatment regimes for treating and controlling the infection. Therefore, understanding the mode of action of antiplasmodial drug used, as well as molecular marker involved in the monitoring of resistance, will help in desiging an effective medicine guideline in all malaria endemic areas and affected countries(101).

Drug Resistance Genetic Markers

Drug resistant molecular markers for disease are determined by genetic alterations that impart parasite resistance to medications employed to treat and prevent malaria(102). Identification of powerful resistant molecular marker is a critical tool for determining the rise as well as dissemination of antiplasmodial medications resistance globally. Genetic crosslink or mapping studies of parasite aided in the identification of the drug resistance genetic marker. Molecular marker detection for drug resistance by using polymerase chain reaction outperforms *in vivo* or *in vitro* tests. The following section discusses the different resistant markers identified for *Plasmodium falciparum* and *Plasmodium vivax* antimalarial drugs.

***Plasmodium falciparum* Chloroquine Resistance Transporter (PfCRT)**

PfCRT protein belongs to the drug/metabolite transporter superfamily as well as the chloroquine resistance transporter-like transporter family. It has 10 putative transmembrane domain that cross the protozoan's digestive vacuole membrane. It is important for parasite survival and major contributor to multidrug resistance.

The Pfcrt gene mutation is essential for identifying CQ resistance as well as its phenotype. The K76T mutation, which takes place in the first transmembrane domain of PfCRT protein and replaces positively charged lysine residue with neutrally charged threonine residue at 76th position, may allow active transport of diprotonated CQ outside the digestive vacuole. Modification in PfCRT protein affects antimalarial drug vulnerability and resistance against quinine, amodiaquine, piperaquine, and lumenfantrine. CQ exhibits cross-resistance agaisnt amodiaquine and quinine primarily mediated by 76T, whereas lumenfantrine dispalys an inverse cross-ressitance having reduced vulnerability in relation with wild type K76. As a result, the K76T mutation in PfCRT is a powerful molecular marker for the antiplasmodial drug.

***Plasmodium falciparum* Multidrug Resistance Protein 1 (PfMDR1)**

PfMDR1 is a protein with transmembrane domain. It has two domains, each with six helical membrane-spanning domains as well as a nucleotide binding fold region, which function as a ATP binding site. Like PfCRT protein, it is found within parasite's digestive vacuole and relates to the ATP-binding cassette (ABC) superfamily. Mutations in the PfMDR1 at N86Y and N1042D codons have been linked to amodiaquine resistance. N86Y means replacement of asparagine with tyrosine at 86th position and N1042D means replacement of asparagine with aspartic acid at 1042th position. K76T or A220S mutations within Pfcrt gene as well as N86Y mutation within Pfmdr1 gene have been related to increased resistance against CQ in field isolates. The term copy number variation (CNV) refers to molecular concept in which genome sequences are repeated or the number of repeats differs between individuals belong to the same species. Furthermore, CNV of Pfmdr1 gene has associated increased resistance to quinine, mefloquine, halofantrine, lumefantrine, or artemisinin.

***Plasmodium Falciparum* Multidrug Resistance-Associated Protein (PfMRP)**

PfMRP is a membrane-spanning protein found on the cell membrane of parasite, encodes for 1822 amino acids. PfMRP is projected to feature two membrane-spanning domain and two nucleotide binding domains, each of which is made up with 6 helical membrane-spanning domains. MRP aids in the transportation of drugs as as well as organic anionic subsrates like oxidized glutathione, glucuronate and sulfate conjugates. Two mutations in PfMRP were discovered to be related with CQ and quinine resitance at position Y191H (replacement of tyrosine with histidine) and A437S (replacement of alanine with serine). When Pfmrp gene was genetically knocked, resistant parasite displayed great sensitivity to varoius antimalarial medications

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including CQ, quinine, primaquine, piperaquie, and artemisinin, whereas higher CQ and quinine accumulation was shown in the sensitive parasite. Therefore, PfMRP is not primarily responsible for determining the drug resistance but rather for altering the antimalarial response to resistance. It is also postulated that PfMRP protein exports numerous metabolites and medications out of the parasite in relation with other transporters.

***Plasmodium Falciparum* Sodium Hydrogen Exchanger (PfNHE)**

PfNHE is a transmembrane protein with 1920 amino acid that is found in the cell membrane of the parasite. PfNHE is expected to contain twelve transmembrane domains. The function of PfNHE has not been entirely understood but it is hypothesized that it participates in active export of protons to maintain the parasite's internal pH around 7.4 in response to acidification by anaerobic glycolysis, the parasite's primary energy source, becoming more acidic.

***Plasmodium Falciparum* Bifunctional Dihydrofolate Reductase-Thymidylate Synthase**

PfDHFR is protein with 608 amino acid. The biosynynthesis of dTMP by thymidylate synthase as well as coversion of dihydrofolate to tetrahydrofolate by DHFR activity are two primary metabolic functions of bifunctional enzymes. PfDHFR is inhibited by pyrimethamine and cycloguanil, therefore decreasing the synthesis of pyrimidine for DNA replication. Resistance against pyrimethamine has primarily been related to point mutation at S108D codon (replacemet of serine with aspartic acid) in the PfDHFR protein. In addition to gene amplification subsequent mutations at N51I (replacemet of asparagine with isoleucine), C59N (replacement of cysteine with asparagine), and I164L (replacemet of isoleucine to leucine) position strengthen their resistance. Double mutation at A16V (replacement of alanie with valine) and S108T (replacement of serine with threonine) position in PfDHFR associated with the resistance of *Plasmodium falciparum* to cycloguanil.

***Plasmodium Falciparum* Dihydropteroate Synthetase (PfDHPS)**

PfDHPS is a enzyme comprising of 706 amino acid and facilitates the interaction of p-aminobenzoic acid (PABA) with pterin derivativen to produce dihyrofolate, a folic acid synthesising starting metabolite that is necessary for pyrimidine synthesis in the parasite. PfDHPS is inhibited by sulfadoxine and dapsone. S436A (serine to alanine), A437G (alanine to glycine), L540E (leucine to glutamic acid), A581G (alanine to glycine), and A613T/S (alanine to threonine/serine) are the five mutations in PfDHPS that have been associated with sulfadoxine resistance in *Plasmodium falciparum*. Higher levels of resistance are associated with mutation at codons 436, 581 and 613, while mutations at codon 437 and 540 make contribution to lower level of resistance. Sulfadoxime is consistently used in conjuction with pyrimethamine, also recognised as SP or Fansidar, because antimalarial drug resistance to monotherapy has evolved. Resistance to SP has been linked to point mutations in both *Pfdhfr* as well as *Pfdhps* genes.

Cytochrome B (CytB)

CytB is a mitochondrial membrane protein with 376 amino acid. It is expected to contain ten putative helical transmembrane domains, which span parasite's the mitochondrial inner membrane. It facilitates the electrons flow across the interior mitochondrial membrane in order to maitain it's electrochemical potential. Mepron binds to the of CytB's ubiquinol binding site, therefore disrupting electrochemical potential and killing the parasite. In *Plasmodium falciparum* only one alteration at Y268N/S/C codon (tyrosine to asparagine/serine/cysteine) within *cytb* gene associtaed with resistance to atovaquone.

KELCH 13 (K13)

The K13 is a protein with 726 amino acid. The C-teminal region of K13 protein contains six kelch motifs composed of beta sheets that are coiled into a propeller domain. It is anticipated that mutation in propeller domain will disturb domain scaffold or change its function. The K13 family protein performs a variety of cellular functions, including protein organisation and interactions. Recently, it was discovered that a point mutation in propeller domain of K13 is a crucial factor in determining artemisinin resistance in *Plasmodium falciparum*.

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Mutations within kelch motif region of K13 protein propeller domain at Y493H (tyrosine to histidine), R539T (arginine to threonine), I543T (isoleucine to threonine), and C580Y (cysteine to tyrosine) have linked to grater resistance to artemisinin. Therefore, polymorphism in K13 protein is a powerful molecular marker in determinig the emergence and dissemination of artemisinin-*resistant Plasmodium falciparum*(101).

Antimalarial Drug Discovery

The challenge with antimalarial drug discovery is identifying new therapies, which are well tolerated in susceptible populations, such as preganat women, children under age of five, and those who are malnourished or coinfected with other infection. Another challenge is determining the best way to treat malaria patient who are asymptomatic while preventing susceptible populations in endemic areas from becoming symptomatic(103).

In addition to compounds that are effective enough for treating symptomatic malaria, medications are able to halt disease transmission, prevent malaria from relapsing due to hypnozoites, safegaurd the most susceptible patients from getting infection and reduce burden of malaria by treating asymptomatic cases of disease(104, 105).

Prioritization of Molecular Targets

It is critical to choose the targets most probably to yield a progressible therapeutic candidate because of high expense of drug discovery or development. It is crtical to identify essential criteria for a drug target. The target must be involved in the progression or pathophysiology of the malaria. This refers to the parasite's cability to persist or spread in case of plasmodium. The target must be druggable, meaning that small molecule or biologic can modify the way it functions. As in instance of the malaria, it must be an orally bioavailable small molecule in order to satisfy the requirement of a low cost of goods.

Target vulnerability is another important concept which describes the extent of duration of how long it modify the activity of target in order to achieve desired phenotypic outcome. Maintaining high degree of therapeutic suppression of target for extended periods of time is difficult. Thus, targets that can quickly "tip" the parasite to death are more desirable. These targets may also require modest levels of supression or a short period of action. Furthermore, malaria patient can soon become very unwell and pass away, the parasite kill rate is critical in case of malaria. As a result, it is not advised to try to treat asexual blood stage infections with targets that need prolonged inhibition to kill a parasite. Slower kill rates may be acceptable for prophylaxis or transmission blocking.

Another critical factor to consider is appearance of drug rasistance, which is a real concern for antimalarials. It is preferable to choose a target that has low tendency towards resistance, or one without clear bypass mechanism. However, understanding mechanism of resistance could result in development of alternate therapies (such as combination of therapies) or efforts for redesigning compounds, for instance to lessen the effect of a frequently occuring resistance mutation.

Selectivity is also required, with a goal of minimising off-target liabilities that could lead to drug candidates failing later in development. Anti-malarial compounds should selectively affect the parasite target rather than host equivalent to avoid toxicological consequences. But its incredibly challenging to predict the latter. Because most drug discovery paradigms rely on screening to find synthetic starting points, hence there is also need to be a way to develop a relevant assay for drug target(106).

Antimalarial Compounds under Clinical Development

The non-profit product development collaboration such as Medicines for Malaria Venture (MMV), along with it's assocites in academia as well as the pharmaceutical firm, has created the whole antimalarial discovery portfolio with assistance of donors (mainly philanthropic foundation and government agencies).

There are two combos OZ439 (referred as artefenomel) with ferroquine (developded by Sanofi & MMV) and KAF156 with lumefantrine (developed by Novartis & MMV). These two combinations are about to enter phase IIb development to assess the effectiveness of single-dose curve and, as well as 2 or 3-day curves in the

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paricular instance of KAF156-lumenfantrine. OZ439 is one of the synthetic peroxide that achieved sustained plasma exposure in humans with a single oral dose. Ferrouine is a 4-aminoquinoline of next genertation with no cross-resistance against chloroquine, amodiaquine or piperaquine. KAF156 is one of the novel imidazolopiperazine. The mechanism of action of KAF156 is unknown, but its resistance marker *Plasmodium falciparum* cyclic amine resistance locus (pfcarl) appears to encode a transpoter on the parasite's endoplasmic reticulum membrane. Notably, while OZ439 and ferroquine primarily act on asexual blood stages, KAF156 also affects asexual liver stage as well as the sexual gametocyte, and thus may influence transmission.

KAE609 (cipargamin) and DSM265 is about to enter phase IIb and are waiting a decision on combination partners. KAE609 is one of the highly potent spiroindole that clears parasites in patients faster than peroxides. It acts through inhibition of PfATP4, and PfATP4 is a transporter present on the plasmodium plasma membrane that is responsible for regulating Na^+ and H^+ homeostasis, and is encoded by parasite's resistance marker. Inhibiting this transpoter, which was found through the sequence analysis of resistant mutants, raises sodium cation concentrations and pH, which provokes parasite enlargement, rigidity and fragility. As a result, in addition to intrnsic plasmodium killing, this also aids host splenic plasmodium clearance in the host. Furthermore, it has been observed that effects on levels of cholesterol in the parasite cell membrane may also contribute to parasite killing by increasing rigidity and hastening clearance of the parasite *in vivo*. DSM265 is a novel triazolopyrimidine that inhibits PfDHODH selectively at both blood and live stages. In humans, DSM265 sustains a blood concentration above the minimum parasiticidal concentration for eight days. DSM265 has shown efficacy in phase Ib trials in therapeutic as well as chemoprotection models in healthy human volunteers.

New compounds are tested for safety and pharmacokinetics in phase I before being tested for effectiveness against asexual blood or hepatic stages of Plasmodium species in healthy volunteers using a controlled infection model of malaria in human. The aforementioned prototype provides a quick and low cost evidence to concept as well as by modifying the dose-response correlation, improves the precision of dosage predictions for future clinical research. The phase I research is ongoing for 2-aminopyridine MMV(390)048 (referred as MMV048), SJ(557)733 (referred as (+)-SJ733) and P218, respectively. Animal experiments show that MMV(390)048 has good exposure, implying that it might be administered in only monodose in conjugation with another medication. SJ(557)733 is a dihydroisoquinoline that inhibits PfATP4 and it is an substitute partner with a totally different structure than KAE609 and has a great preclinical safety and development prospects. P218 is currently undergoing evaluation in humanoid malaria infection cohorts under controlled condition.

Eight more molecules are active in preclinical testing. Four of these molecules are replacements for the leading molecules that act on established mechanism: the aminopyrazole PA92 (called as PA-21A092) as well as thiotriazole GSK030 (called as GSK3212030A) both act on PfATP4. DSM421 is one of the triazolopyrimidine substitute for DSM265 and UCT943 (referred as MMV642943) is a substitue for MMV(390)048. Three molecules exhibit novel mode of action and resistance markers. M5717 (referred as DDD498/DDD107498) inhibits *Plasmodium falciparum* elongation factor 2 and thus protein synthesis. M5717 is effective against plasmodium at all stages of it's life cycle. MMV253 (called AZ13721412) would be rapid-acting triaminopyrimidine and has a V-type ATPase as a marker of resistance. The novel oxaborole AN13762 (known as AN762) has a novel resistance marker. Collaborations with MMV have resulted in the development of all of these compounds.

JPC3210 (MMV 892646) is the eighth compound under preclinical studies, led by Jacobus Pharmeceuticals. JPC3210 is a newer aminocresol that outperforms the prevoius candidate (WR194965). WR194965 was developed by the Walter Reed Army Institute of Research and evaluated in patients during the development of mefloquine as in the 1970. MMV 892646 has an unspecified mode of action but exhibits potent, long-lasting effectiveness in preclinical models, implying that it could be used in a single dose for both prophylaxis and treatment(2).

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Recently, FDA-approved antifungal drug griseofulvin has been shown to prevent cerebral malaria in mice. Griseofulvin targets parasite heme synthesis and serves as an adjunct therapy for treatment of cerebral and severe malaria(107).

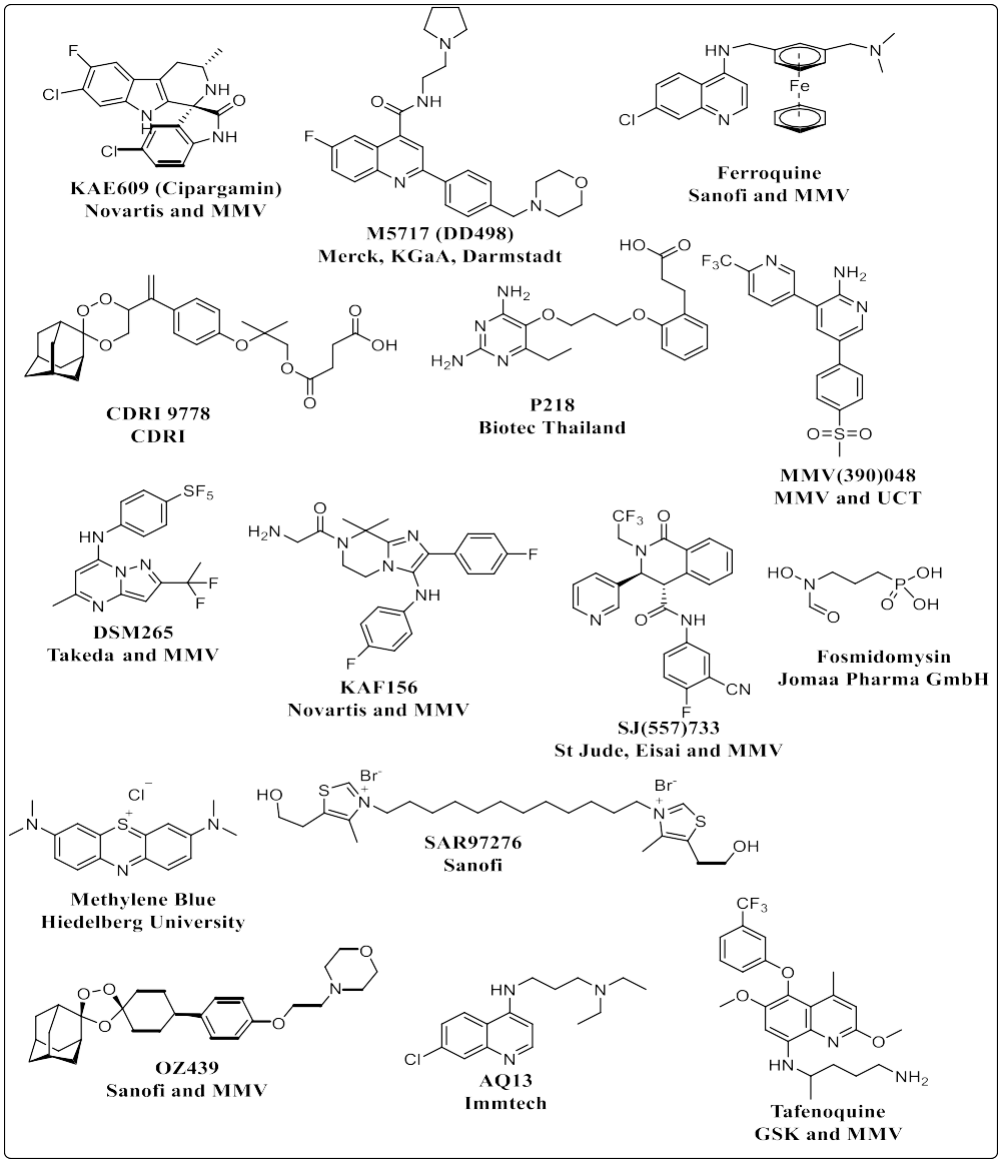

Figure 6 Structure of anti-malarial compounds under clinical development

Malaria Vaccine Development

The complicated antigenic makeup and complex life cycle of parasite make develoing a vaccine for the disease a difficult and challenging endeavour. Development of vaccine to malaria is a major area of reaearch since it has potential to save billions of deaths worlwide. The currently available techniques are not enough for malaria eradication. Despite extensive research being done over the past couple of decades and several vaccine reaching clinical trials, none of them are currently being used in the clinical practice because of insufficient immunogenicity. Despite the fact that vaccines for parasites are currently being developed, there is currently not any FDA-approved vaccine for parasite species more complicated than viruses and bacteria(108).

Scientific challenges in vaccine development

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Lack of understanding of parasite is a signifiant obstacle to the vaccine development. It takes extensive, time-consuming and challenging lab or field studies to understanding the structure as well as antigenic variation of parasites population. Another significant scientific obstacle is the parasite polymorphism and antigenic variation. Unfortunately, there are not examples of malaria immunity and nature, and many of the programs to develop vaccine are based on naturally accquired immunity. It is challenging to understand why some people are protected while others are not because mechanism of immune system is yet unknown. Insufficient animal models and confusion in the definition of predetermined objective create difficulties in identifying the optimal technique for developing a vaccine to malaria. Eventhough in specific animal disease model with established results, there is mostly uncertainity in transferring the success of protection as in model systems to success in humans.

Recombinant proteins, gene-based (DNA and viral vector) vaccines, attenuated whole organisms, peptides, and prime-boost startegies are all used in the development of vaccine. Prime-boost strategies combine various antigen delivey system that encode same epitopes or antigens with various adjuvants. Studies from the 1960s showed that immunization to radiation-attenuated sporozoites in monkey and experimental rodent models resulted in species-specific as well as strain cross-reactive protection. Positive protective immunity levels are shown by studies. However, the participants who had received numerous *P. falcipaum* malaria strain vaccinations were not immune to *P. vivax*. The experimental hosts immunised with attenuated sporozoite vaccine as well as the protected volunteers' sera cells were used to identified target antigens. Circumsporozoite protein, the first parasite protein to be successfully cloned or sequenced in *P. knowlesi* as well as *P. falciparum*, was the first antigen to be discovered through serological screening. It contributes significantly to protection. The immunoglobulin and cells demonstrated varoius roles in several malaria species and strains whenever sporozoite was radioactively treated in the rodent models. Even though, there are many different cellular reactions visible, the interferon produced by immune system thought to act on intracellular hepatic exoerythrocytic forms. When the vaccinations demonstrate a multipronged strategy, the antibody kills majority of the highly infectious sporozoite inoculum. The cellular responses specifically attack on intracellular exoerythrocytic forms by using inhibitory cytokines or direct cytotoxicity(109).

Vaccine targetable process within plasmodium life cycle

There is no vaccine for malaria at the moment. In the past 30 years, attempts to develop vaccines have focused on pre-erythrocytic (PE) atage (sporozoite and liver stage), blood stages and sexual stages. Malaria could be prevented with a fully effective PE vaccination by preventing the development of blood-stage infection. To reduce control morbidity and mortality, vaccine that target asexual blood stage that is actively reproducing have been considered crucial. Vaccines against the sexual phase would halt the transmission cycle but they would have no direct impact on an infection that has already taken hold in the vaccine.

Perhaps, a malaria vaccine could act at various stage of life cycle. The parasite population dynamics in each host is represented by panel on the left.

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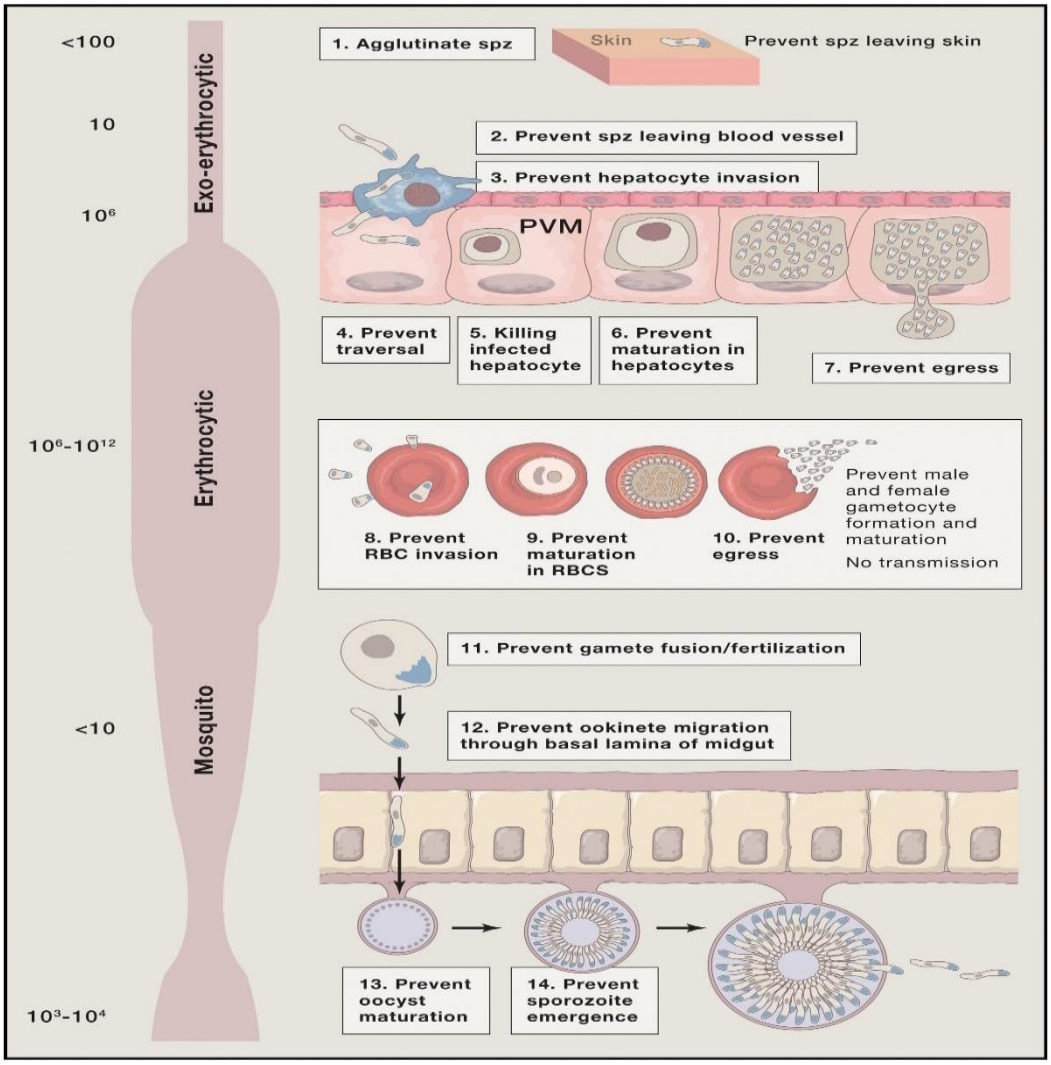

Figure 7 Vaccine-Targetable Processes withhin the Malaria Life Cycle

As the idea of elimination gained popularity, the PE and erythrocytic stages became the focus of vaccine development. In contrast to targeting replicating erythrocytic stages, which are more numerous or have developed a range of immune evasion mechanism for long term survival, injecting soprozoite earlier to hepatic invasion and mosquito's midgut epithelial transmission stages will boost vacination efficacy. As the immunity produced towards these targets seems to be totally stage specific, the concern with this approach is that if one sporozoite or ookinete manages to spill through, the pathogenic replicative cycle will continue (110). This will become progressively more crucial as we get closer elimination. A combination of declining conditions for a resurgence malaria transmission andpopulation declines in naturally acquired blood-stage immunity.

Malaria Vaccine under pre-clinical development and clinical trials

The use of several antigens from multiple stages of plasmodium life cycle, is current focus of vaccine development. The perfect vaccine should provide protection against both *Plasmodium falciparum* and *Plasmodium vivax*, which should have long lasting, protective efficacy of at least 75%.

The most advanced contender is RTS,S invented by GalxoSmithKline and the Program for Appropriate Technology in Health Malaria Vaccine Initiative under the trade name Mosquirix. RTS,S is made up of a transgenic protein containing components of the *Plasmodium falciparum* circumsporozoite protein, hepatitis B virus surface antigen as well as a proprietary adjuvant. In the first year after

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vaccination, RTS,S cut the number of disease cases by half in 4358 children 5-17 months, preventing 1774 cases for every 1000 chlidren due to herd immunity. RTS,S showed effectiveness of forty percentage over the course of 48 months in those children who got four doses of vaccination over a four-year period. When children only got three doses of vaccination, the effectiveness of RTS,S dropped to 26% over the course of entire follow-up period. Thus, RTS,S vaccine was unable to offer long term protection.

Whole-cell sporozoite (PfSPZ) vaccine is radiation-attenuated vaccine and provides sterile immunity (no erythrocytic development) to homologous challenge (injection to sporozoite of the same parasite strain) in preliminary phase I/IIa clinical trials(111).

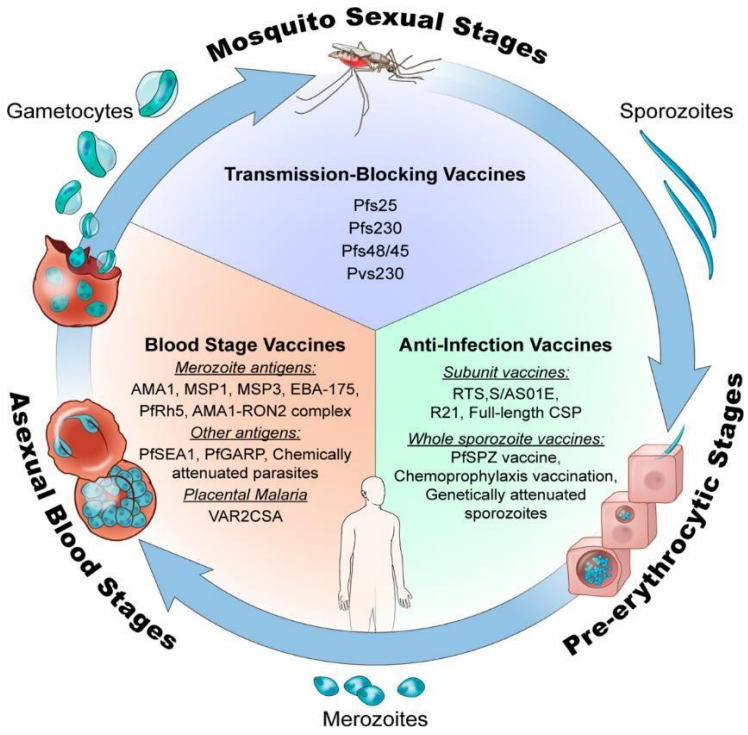

Figure 8 Vaccine candidate under pre-clinical and clinical trial

Moreover, PfSPZ has significant drawbacks, such as deficiency of proven heterologous (cross-strain) protection, the need of intravenous administration and high parasite load to stimulate anti-sporozoite immunity within volunteers, as well as logistical need for compressed nitrogen cold chain to preserve viability of the vaccine. Some living cell sporozoite vaccine approaches, including genetic attenuation of parasites that may be valuable in regards to quality control and CPS(chemoprophlyaxis with sporozoites), where fewer pathogens are required to stimulate immunity but require CQ treatment after each immunisation dose, may also have gradual advancements over PfSPZ but then all end up sharing the fundamental problem of inducing strain-transcedent protection, a top highest for vaccine development(112).

The most difficult challenge to effective vaccine design is Plasmodium's complexity. Researchers have defined some important parameter that should be considered during vaccine development. These are safety, efficacy, immunogenicity and methdology adopted for testing new malaria vaccines.

4. Conclusion

The malaria eradiaction programme started in 1950s but failed globally due to resistance of mosquitoes to insecticides, resistance of parasite to drugs and

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administrative issues. Resistance to all anti-malarial medicines has been reported. Develpment of new antimalarial compounds and vaccine is one of the approach to fight against malaria and drug resistance. Therefore, improvement in our knowledge of the fundamental molecular pathway of pathogenesis of malaria is significant. As a result, research into new antimalarials and potential vaccine is still ongoing. There are many drug and vaccine candidate under clinical development. To ensure eradication of malaria, specific and critical actions are required at national, regional and global levels.

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