Chapter

Application of Radiation Technology: A Novel Vaccine Approach to Induce Protective Immunity against Malaria Infection

Nikunj Tandel, Devang Trivedi, Aditi Mohan Krishnan and Sarat Kumar Dalai

Abstract

Among the numerous infectious diseases, malaria remains a major health challenge. Despite the various approaches adopted for the vector control and availability of antimalarial drugs, the success of malaria eradication is dampened by the spread of drug and insecticide resistance, unavailability of proper diagnostic treatment and successful vaccine. Among the various approaches, vaccination with the aim of developing protective immunity is the most suited, safe and reliable approach for the entire mankind. Numerous approaches are in use for vaccine development; however, they suffer from the drawbacks that immunity developed is short lived and are both species- and stage-specific. Of late, radiation sterilization has drawn the attention in the vaccine development due to its advantages over the conventional methods, and successful clinical trials of irradiated vaccines against the pathogens and tumor. Recently, a novel approach of genetically attenuated sporozoites (PfRAS, PfSPZ, PFSPZ-GA1 sporozoites vaccines) has shown promising results by generating protective immunity against the homologous and heterogenous infection in the clinical trials. Radiation techniques have also been beneficial in controlling the insects by sterility technique. In this chapter, we have recapitulated the role of radiation biology in the malaria vaccine development with its current status and future challenges associated with the development of radiation attenuated parasite vaccine.

Keywords: malaria, infectious disease, vaccine, radiation technology, immunity, genetically attenuated sporozoites

1. Introduction: basic malaria biology

Malaria, an ancient disease has been found recorded in several historical documents and the word malaria comes from the Italian mal’aria meaning spoiled air [1].
Ionizing and Non-ionizing Radiation

It is a vector born disease caused by the parasite, belonging to genus *Plasmodium* and out of five different species, the burden of malaria leads by *Plasmodium falciparum* followed by *Plasmodium vivax* in humans [2]. 219 million cases of malaria were reported in 2017 with the death toll of 4,35,000 and the latest world malaria report suggest the stalled in the progression of the reduction in malaria elimination across the globe [3].

Almost half of the world population is at the risk of malaria, with more than 85% cases in sub-Saharan Africa followed by South-East Asia, West Pacific and others [3]. The population groups are at higher risk of malaria, and developing fatal severity include infants, children under 5 years of age, pregnant women, immunocompromised patients, non-immune migrants, mobile populations and travelers [4, 5]. In areas where malaria transmission rate is high, among these, children under 5 years are particularly receptive to infection, Illness, and death; more than 2/3 (70%) of all malaria deaths occur in this age group [4]. The number of under age of 5 years for malaria deaths has declined from 4,40,000 in 2010 to 2,85,000 in 2016, nevertheless, it remains as a leading reason for the death under 5 years children, taking the life of a child every 2 minutes [3].

1.1 Malaria life cycle

*Plasmodium spp.*, a Causative parasite for malaria exhibits a complex life cycle that switches between *Anopheles* mosquitoes (vector), and invertebrate hosts that form unique zoite formation to evade different cell types at specific stages [6, 7]. It comprises of three different types, two of these (asexual stage) in vertebrate which are exo-erythrocytic cycle (liver/asymptomatic stage) and erythrocytic cycle (blood/symptomatic stage). And the third phase (sexual stage) is in the mosquito that is sporogenic cycle (infective stage) [7].

During the probing for the blood meal using its proboscis on the host, the infected female mosquito injects sporozoites (SPZs) within its saliva into dermis of uninfected human [8, 9] which contains vasodilators and anticoagulants that facilitate the ingestion of blood [10]. Most of the parasites reside in the skin between 1 and 6 hours [7, 8]. Many fail to migrate to the lymph nodes (LNs) and approximately 20% of them migrate directly into the skin-draining LNs through the lymph [11, 12]. It triggers the induction or modulation of the host immune system followed by an antiparasitic immune response [13, 14]. On the other hand, a small proportion of SPZs randomly transverse to the nearest blood vessel [12]. After crossing the endothelial barrier of the skin, the SPZs enter to the circulation and reached to the liver which is critical for the infection cycle as the development of merozoites occurs [15–18].

The traversal activity of SPZs through different host cells and molecular events that underpin the transformation of SPZs into merozoites via erythrocyte invasion involves the expression of thousands of proteins [7]. These SPZs migrate via blood flow to the liver retained by hepatic stellate cells (HSC) and glide, further using the Kupffer cells as a shield, traverse the liver sinusoidal endothelial cells (LESC) barrier in a sinusoids [8]. It has been moving in several hepatocytes through the space of Disse by the thrombospondin related anonymous protein (TRAP) and circumsporozoite protein (CSP) interactions before actually infecting one of them to form a parasitophorous vacuole, that aids in protecting from the host’s immune system and provides nutrients for the SPZs to replicate and differentiate into merozoites [8, 10]. The healthy or successful sporozoites release up to 10,000 to more than 30 to 40,000 merozoites per hepatocyte into the bloodstream within parasite-filled vesicles called
merosomes through a process of budding which use the host-cell membrane to escape the host immune system (Figure 1) [19].

The merozoites release in the hepatic circulation that invades erythrocytes in a short duration via dynamic and multi-step process that includes pre-invasion, active invasion, and echinocytosis [21] through the involvement of proteins named merozoite surface protein (MSP) [7]. The blood stage is symptomatic stage that develops once the erythrocytic cycle produces a parasitemia above a certain threshold approximately 50–100 parasites/μL (microscopy). The merozoites released into the blood reinvade the new red blood cells (RBCs) and continue to replicate or in some instance, they differentiate into male and female gametocytes [22, 23]. Mature gametocytes travel throughout the body and deposited mainly in skin capillaries from which they are taken up by the mosquito for the next blood meal. Once they reach to the mosquito gut, male gametocyte produces eight microgametes with three rounds of mitosis, meanwhile female gametocyte mature in macrogametes in the gut of the mosquito [24]. The fusion of the male and female gametocyte forms a diploid zygote that elongates into an ookinete; which exits the epithelium through the lumen of the gut as an oocyst and undergoes cycles of replication and forms sporozoites. It moves from the abdomen and resides into the

Figure 1.
The basic malaria life cycle of Plasmodium falciparum (adapted from [20] with permission).
salivary gland followed by the next blood meal of mosquito during which it will inject the sporozoites to the healthy host [24].

1.2 The liver stage infection of malaria parasites: yet hold promises

The liver is the most important organ and known for its role in blood purification, detoxification of chemicals and metabolizes of drugs, role in the immune system and homeostasis [25]. This unique architecture of liver favors the interaction between leukocytes and hepatic cells and intra-hepatic recruitment of T cells which recognize their cognate antigen within their vicinity [26]. Among the multi-stage life cycle, liver/exoerythrocytic stage of the malaria parasite is least known due to several reasons, however recent developments of humanized mice have revealed the importance of liver stage [2]. All the same, liver stage parasites in murine and humans evade the immune clearance despite the presence of antigen presenting cells (APCs) [8]. Cytotoxic T cell (CD 8$^+$ T cell) requires to kill the intracellular parasite infected cell as they are intracellular organism reside in the hepatocytes. Therefore long lasting immunity, reduce/remove the parasitic load in asymptomatic exoerythrocytic stage and to prevent their liver to blood stage transitions are the main concern for any malarial parasitologist to develop and improve the vaccine strategies.

1.3 Current status of antimalarial therapy and standing of vaccine initiative

To prevent and eradicate malaria, prophylaxis that has no adverse effect and versatile for all is an urgent requirement. Till now various antimalarial therapy of drugs, insecticide-treated bed nets and different diagnostic tests [27] are in use, however the success of malaria infection halted by the insecticide [28, 29] and parasite resistance [28, 30, 31]. This life-threatening condition needs the novel antimalarial therapy that can control parasite at different stages of its life cycle (Figure 2).

![Figure 2](adapted and modified from [32]).
Nowadays, a novel approach of combinational drug therapies are in use such as Artemisinin based combinatorial therapy (ACT) and others though, the success rate is low or restricted to the area due to the diversity among the parasite [33]. Despite the availability of drugs from natural resources or synthetic compound, the parasite still surpasses and escape the host immune system as well as dampen the effect of the drugs [34]. Also, the recent finding has alarmingly shown that parasite has been gaining resistance against artemisinin and its derivatives [31, 35]. In this bleak condition, the malaria vaccine is urgent and indeed. Different approaches are in practice for the development of malaria vaccine based on immunogenic peptides, usage of mosquito and/or parasite antigens, usage of adjuvants, radiation or genetically modified sporozoites and so on [36]. Different experimental evidences shed the light on the importance of novel vaccine approaches (attenuated whole sporozoites) which strongly confirms the role of APCs resides in the liver capable to recruit CD8+ T cells together with the cytokines (IFN-γ and TNF-α) required for the sterile protection [21, 37–43]. Despite the sterile immunity last longs for the 6–9 months, a recent study confirmed the usage of the intermittent challenge of radiation attenuated sporozoites in mice stay long lasting up to 18 months and give rise to sterile immunity [44]. The approach of radiation for protective immunity has gained attention in the scientific community to eradicate the morbidity and mortality of malaria infection.

2. Radiation and its impact on health

Radiation is a form of energy which travels and transmits or emits from its source as a wave (ionization radiation) or in the form of electron particles (non-ionizing radiation) [45]. The broad range of electromagnetic spectrum consists of harmless radio and microwaves, sunlight includes longer (infrared) and shorter (ultraviolet) wavelength and finally the higher energy specific wavelength of X-rays and gamma rays which exist the electrons from the atoms through the ionization process [45, 46]. During the process of radioactive decay there are mainly three types of ionizing radiation emitted; alpha, beta and gamma rays. Other than this, X-rays can be occurred naturally or produced by machines [47]. The major difference between this ionizing radiation (X-rays and gamma rays) is the photon’s energy of individual rather than the energy of the total dose of the radiation alongside their source of origin [48].

As the entire world is in the vicinity of the radioactive environment, we all, more or less, are exposed to a certain level of background radiation [49]. It has been reported that more than 80% of human-radiation doses are uncontrolled which are mainly consist of the natural sources, terrestrial and exposure through inhalation or intake of the radiation (mainly through medication) [50].

There are more than 60 natural radionuclides are found in the environment and no place on the earth is without spontaneous radiation activity. It is mainly observed in the soils and rocks (uranium and thorium decay), in water sources such as naturally occurred lacks, rivers and oceans and Human-made buildings and homes [51]. Also, the effect of gamma radiation on natural radioactivity and their associated exposures mainly depends upon the geological and geographical location together with their appearance at different levels in the soil of the respective the region [50]. Overall, we received an average dose of 2.4 mSv/year (mSv stands for millisieverts, one-thousandth part of a sievert, an SI unit and currently used in the radiation protection standards) from the background radiation which may vary from 1 to 10 mSv/year and rely on the location of the region; however it
can exceed above the 50 mSv/year. Therefore, if an average of 2 mSv/year of the radiation dose exposed to an individual, the person at the age of 80 years will be accumulated almost 160 mSv radiation originated from the natural sources [50].

2.1 Radiation biology

The term radiation biology came across into the picture during 1963 when Bergonie and Tribondeau assumed and stated that the immature, undifferentiated and continuously dividing cells are more prone towards the radiation with compare to the cells which are fully matured, differentiated and not actively participated in the cell division process [52]. Therefore, the radiosensitive cells such as stem cells, the stratum basal of the skin and stomach mucosa; continuously experiencing cell division (mitosis) and exhibits certain effects after the exposure towards the ionizing radiation resulting into the cell death or cell injury. Contradictory, the radioresistant cells such as neurons which never divides or do it very slowly show less inclined towards the cell injury or death after the radiation exposure [53]. The experiments carried out on fruit flies and mice have shown the effects of radiation as mutation was occurred however, it was notified that all the mutation were similar to the spontaneously generated one. Also, it was linked to the dose and exposure rate of the respective ionizing radiation [54].

2.1.1 Interaction between radiation and human cells

The interaction of human cells and radiation is just the likelihood and therefore, any permanent damage occurs to the tissues is not due to the facing off them each other during the cellular repair mechanism [53]. The processes of energy deposition have not any signature pattern (very rapid, $10^{-18}$ s) and the interaction takes place at the cellular level affect the organ as well as the entire system [45]. Alongside, there is no established cellular damage associated with the radiation, and heat, chemical or physical damage is also accounted for the same. The destruction occurs as a result of radiation towards the cells has the latent period followed by observable responses. The latent period remains for a prolonged time in case of low radiation whereas, it accounts for minutes to hours for the higher dose of radiation and the radiation biology entirely rely on these basic principles [53, 55].

The interaction of ionizing radiation with the cells, possibly occur through any of the two ways; direct interaction within the cells hit the macromolecules (proteins and DNA) resulting into the death of the cells or the mutation of the DNA. This mechanism can happen during the higher doses of the radiation as the cellular repair mechanism is tightly regulated [53]. Another one is the indirect pathway where the radiation energy is trapped inside the cellular compartment and interacts with the water rather than macromolecules followed by hydrolysis of water produced the free radicals inside the cells [53, 56]. This will lead to the loss of the important enzymes resulting in the cell death or the mutation. As the result of an interaction, there are mainly three types of cellular injury arises: (1) delayed division, (2) failure of the reproduction and (3) death during the interphase of the cell cycle [53].

The intensity of the damage occurs inside the cells rely upon various parameters including the types and its source, duration, doses, exposure and its energy [57]. The knowledge about the risk of the radiation has been studied, documented and referred well from the survivor of the atomic bombs at Hiroshima and Nagasaki in the Japan during the Second World War. Also, the additional inputs are given by the studies conducted on the radiation industry workers [45].
2.2 Radiation therapy: application in medical research

It was during the 1895, when Wilhem Roentgen has invented the X-rays to see the things visibly inside the body without surgery. This historic discovery has transformed the medical field and currently, it has been widely used to diagnose the injuries and diseases [45]. At present, more than 50% of our exposure contributed to the medical sources lead by X-rays and CT scan. Nowadays, understanding the radiation risk and their effects at molecular, cellular and organ level is mainly

![Diagram of radiation therapy and its effects on tumor cells.](image)

**Figure 3.** Radiation therapy and their different consequences can be used for antitumor combination therapy for cancer therapeutics (adapted from [62] with permission).
studied for the diseases. This will bridge the gap and guide the health physicist to determine the safety level of the radiation to use in the medical, industrial and scientific world for the betterment of the mankind [45, 49, 58].

Despite the hazardous effects of radiation on the cells, if the cell death mechanism is instinct or properly targeted then it will be the achievement in the medical field for the cancer patients where it is using as radiation therapy [59]. It still remains as the preliminary cancer treatment and more than 50% of all the cancer patients received radiation therapy and stands for 40% of the curative treatment [60]. It is believed that it can evoke the tumor-specific immune response to destroy the tumor cells as well also travel to the site of the disease [61].

Recent research confirms the potential usage of radiation therapy in cancer therapeutics by converting tumor to favorable condition and makes them immunostimulatory milieu [62]. Experiential data suggest that the combination of radiotherapy alongside the immunotherapeutic agents can produce the synergistic effects (Figure 3) [61, 62]. From all the above-stated information, it has become clear that recent developments in radiation therapy [61, 63, 64] may have proven to be the most critical part in the successful development of cancer vaccine.

2.2.1 Radiation and vaccine development

Usage of chemical and physical methods to develop a stable and safe vaccine is the gold standard method through which the pathogen will be converting into the inactive form. Despite the successful application in various medical conditions to eradicate the disease or to develop the sterile immunity; the fast, reliable and rapidly generated vaccine is required [58]. Recently, radiation sterilization is been under the process for the vaccine development which surpasses the chemical or other types of contamination. Also, it destroys the nucleic acid of the respective pathogens through penetration without disturbing the cell surface antigens. It is been in under the clinical trials for the various types of cancers [65–67] however, due to the issues of safety and other regulatory affairs it is under development at industrial level [58]. On the other side, results of current clinical trials of using the various irritated vaccines for numerous pathogens and tumors has attracted the researcher and scientific world towards the preparation of various radiation-based vaccine for various infected and non-infected diseases [61, 62, 64, 68]. The ongoing effective development of irradiated vaccines for malaria and influenza have exhibited the attainability of this approach, and have shown the promising results alongside the advantages of the radiation therapy by overcoming the limitation of existing facilities without using any sophisticated technological approach [69, 70].

3. Role of radiation therapy in malaria vaccine: developments and challenges

Malaria vaccine development is an active research area with enormous challenges. An effective vaccine for P. falciparum is needed in malaria-endemic populations; however, none of the licensed malaria vaccines and candidates consistently produced long-lived protection [71, 72]. The malarial parasite undergoes continuous morphological changes and displays antigenic variations during the entire life cycle in both the host [73]. As a result, parasite evades the protective immune responses of the host and long-term protective immunity is not observed in malaria-infected individuals [74, 75].
*Plasmodium falciparum* has a multi-stage life cycle and a large 23 Mb genome expressing 5268 putative proteins. Many of these proteins exhibit allelic variation between species or antigenic polymorphism typically at sites recognized by antibody or T cell responses [76]. To date, only a handful of the nearly 5300 potential target antigens expressed by *P. falciparum*, representing less than 0.3 percent of the genome, have been pursued as vaccine targets [76, 77]. From the host perspective, malaria is a chronic infection and the *Plasmodium* parasite is capable of evading or modulating the host immune response. Based on the life cycle of the malaria parasite and the process of infection, malaria vaccines are divided into four potential target groups; interruption of human to mosquito transmission (parasite sexual and mosquito stages), inhibition of clinical consequences (asexual blood stage), prevention of mosquito to human transmission, and pre-erythrocytic infections (sporozoite [SPZ]/liver stages) [78]. As per the recent advancement in the malaria vaccine development mainly three types of vaccine candidate targeting different stages of malaria parasite have been intensively investigated named as pre-erythrocytic vaccines, blood-stage vaccines and transmission-blocking vaccines [79, 80].

### 3.1 Pre-erythrocytic vaccines

The pre-erythrocytic stage does not cause clinical disease, and there is no convincing evidence for naturally acquired protective immunity to this stage in individuals living in malaria-endemic areas [81]. Thus, this stage would appear to be an unattractive vaccine target. Nonetheless, the most advanced vaccine in development is a protein expressed at this stage that covers the parasite surface, the circumsporozoite protein (CSP). One of the most advanced anti-malaria vaccines at a clinical level is the RTS,S/AS01, a subunit vaccine consisting of the *P. falciparum* CSP fused with the hepatitis B surface antigen (HBsAg) [82–85]. Although this vaccine did not appear to elicit a CD8+ T cell response, CSP-HBsAg induced a specific CD4+ T cell response targeting the whole SPZs [86, 87]. The mechanism by which RTS,S confers protection against the blood-stage disease remains poorly understood. It seems that RTS,S induces protection against clinical malaria by temporarily reducing the number of merozoites emerging from the liver [69]. This may allow prolonged exposure to subclinical levels of asexual blood-stage parasites, therefore boosting the naturally acquired blood-stage immunity.

### 3.2 Blood stage vaccines

Blood-stage vaccines work on the principle of anti-invasion and anti-disease responses by blocking the invasion of erythrocytes by merozoites and preventing malarial disease [88]. The extensive genetic diversity of the parasite and the selective pressure are factors to be considered in the development of effective blood-stage vaccines. At present, several blood-stage antigens are in clinical trials: apical membrane antigen 1 (AMA1) [89], erythrocyte-binding antigen-175 (EBA-175) [90], glutamate-rich protein (GLURP) [91, 92], merozoite surface protein (MSP) 1 [93], MSP2 [94], MSP3 [95–97] and serine repeat antigen 5 (SERA5) [98]. All these antigens are highly expressed on the surface of merozoites. During the blood-stage vaccine development various assay such as ELISA, western blot and immunofluorescence assay, invasion inhibitory assay, antibody-dependent cellular inhibition (ADCI) assay, phagocytosis/opsonization assay, T cell-based assay and other antibody-based assays are in use for their screening of the candidate vaccine [99].
### 3.3 Transmission-blocking vaccines

Transmission-blocking vaccines (TBV) target antigens on gametes, zygotes and ookinetes to prevent parasite development in the mosquito midgut [88, 100]. The aim of these vaccines is to induce antibodies against the sexual-stage antigens to block the ookinete-to-oocyst transition to stop the subsequent generation of infectious sporozoites thereby acting as important tools for protection against epidemics [101, 102]. The leading vaccine candidates in this group include the *P. falciparum* ookinete surface antigens Pfbs25 and Pfbs28 and their *P. vivax* homologs Pvs25 and Pvs28. To improve the immunogenicity, Pfbs25 was expressed as a recombinant protein that was chemically cross-linked to Exoprotein A and delivered as nanoparticles [103]. This enhanced the immunogenicity of the vaccine in mice, and it is currently undergoing Phase I trials in humans. Because the antigens are never naturally presented to the human immune system, one of the potential limitations of the TBV approach is that the absence of natural boosting following immunization might limit efficacy [88]. The vaccine would confer no protection to the vaccinated individual unless combined with an effective pre-erythrocytic or erythrocytic vaccine. Nevertheless, TBVs could be important tools for a malaria elimination and eradication program, for prevention of transmission of the disease [88].

### 3.4 Novel usages of radiation attenuated sporozoites (RAS) and effects on the host immunity

A recent landmark finding that set the standards for immunological protection against malaria infection was established by immunization with irradiated sporozoites [82, 104]. Because the parasite undergoes morphological changes and displays antigenic variation at each stage of infection, whole parasite vaccines have an advantage [105–107]. In the early 1940s, Russell and Mohan [104] first demonstrated that inactivated *P. gallinaceum* SPZs provided protection against challenge with infectious *P. gallinaceum*. In 1967, Nussenzweig et al. [82] reported that a killed *P. berghei* sporozoites (SPZs) vaccine was unsuccessful, but that an X-ray irradiated SPZ vaccine provided significant protection in an SPZ-challenge mouse model.

In the 1970s, researchers showed that immunizing human volunteers with bites from irradiated mosquitoes carrying *P. falciparum* SPZS (PfSPZ) or *P. vivax* SPZ (PvSPZs) provided protection against challenges with infectious SPZ [108–112]. Because infected mosquitoes cannot be used for immunizing large numbers of individuals, a team at the Vaccine Research Center, NIH developed an injectable and cryo-preserved irradiated PfSPZ vaccine that met the vaccine regulatory standards [58, 113]. The group succeeded in raising mosquitoes on an industrial scale to good manufacturing practice (GMP) levels and harvested large amounts of PfSPZ from the mosquito salivary glands. Although studies provided proof of concept that sporozoites could induce high-level immunity, as a vaccine for human use, PfRAS immunization was deemed impractical for many decades due to the complexity of administering a vaccine through natural way of mosquito bite, the requirement for a secure vivarium and a laboratory for maintaining *P. falciparum* in culture, and the perceived need for five or more immunization sessions to achieve a sufficient number of bites. Recently, it has been demonstrated that the Sanaria PfSPZ vaccine is safe, well tolerated, easily administered by syringe using a variety of routes, and can induce 100% protective efficacy against controlled human malaria infection (CHMI) when administered intravenously [109, 114].
The sterile immunity induced by RAS appears to be mediated primarily by CD8+ and CD4+ T cell-dependent mechanisms targeting antigens expressed by sporozoites and liver-stage parasites [115–118]. Antibodies also appear to contribute to protection. Studies in mice and humans show that immunization with RAS induces sporozoite-neutralizing antibodies that recognize the CSP, an abundant protein forming the surface coat of the sporozoites [109, 119, 120]. This finding led to the cloning of *P. falciparum* CSP and the formulation of several CSP-based sub-unit vaccines designed to induce protective antibodies [121, 122]. Although efficacy was low, subsequent development of CSP using a particle-based approach has led to the currently most advanced malaria sub-unit vaccine, RTS, S/AS01, that elicits 30% protection in young children [123] primarily mediated by anti-CSP antibodies and CD4+ T cells [124, 125]. The partial efficacy of these first generation subunit vaccines suggests that a better understanding of RAS-induced protective mechanisms may provide a rationale to develop alternative or improved subunit strategies using newly discovered antigens or more potently inducing cell-mediated immunity. Despite all difficulties, the most convincing evidence that vaccination against malaria is feasible has come from experimental studies in rodents, monkeys and human subjects in which attenuated sporozoites induced sterile protective immunity.

### 3.5 Current status and future development: role of radiation in malaria vaccine development

The first whole sporozoites vaccine (WSV) studied in both rodents and humans were RAS and also the first to confer protection in humans when administered by mosquito bites. A major advance for vaccine development was the ability to isolate a purified, aseptic, and cryo-preserved product of RAS for clinical trials (PfSPZ vaccine). A subsequent scientific advance showed that intravenous (IV) administration of PfSPZ vaccine was required for inducing potent immunity in humans [109]. These studies provided the first evidence for using IV administration of a preventive vaccine in humans. More recent results with PfSPZ vaccine from sub-Saharan Africa reported that PfSPZ vaccine was well tolerated, safe, and easy to administer by direct venous inoculation (DVI) of healthy volunteers in the clinical trial setting. The proportion of participants with any infection from 28 days after the fifth vaccination to the end of the malaria season (20 weeks) was lower in the vaccinated group than in the control group [126]. These data suggest that WSV can provide some protection against malaria infection during intense transmission (93% infection rate among placebo) [79]. Recent progress in manufacturing whole organism vaccines has prompted several vaccinology questions: (1) Can whole sporozoite vaccines be improved? (2) What are the optimal doses, routes of administration, and adjuvants that confer sterilizing immunity? (3) Can other antigens be included? (4) Are we exploring all protective immune mechanisms? (5) Does efficacy differ in populations from endemic versus non-endemic areas? (6) What is the impact of antigen polymorphisms? [80].

The future lies in improving dosing strategy, immunogenicity enhancement, and/or alternative vaccine approach for populations in malaria-endemic regions. To halt the spreading of the malaria infection is not only the development of the vaccine against it, however; the mosquito biology equally holds the importance in understanding the malaria pathophysiology and parasite biology which may be understand more in detailed by radiation biology [127]. Combination of different approaches based on attenuated whole organism sporozoites may be a valuable tool that would help unearth
host mechanisms of protection as well as surrogate measures of immune protection for malaria, thus providing crucial leads towards discovery of long-awaited vaccine [128].

4. Conclusion

Malaria is one of the most fatal diseases in the world related to humans in terms of morbidity and mortality. The asexual blood stage \( P. falciparum \) culture \textit{in vitro} was successfully achieved in the early 1980s. However, various aspects related to their life cycle are unclear. The technological advancements in the field of epidemiology and entomology supported the research not only in reducing the burden but also allow scientists to go one step further in malaria parasitology by the staining the different stages of the parasites. The reports of the emergence of drug resistance in last decade against the various \textit{Plasmodia} strains resulted in the serious condition to find out the causes. Given this bleak situation, the need to develop additional control measures, such as the malaria vaccine, is indeed. Understanding the mechanism of protective immunity to natural infection is critical in engineering an effective vaccine. The usage of radiation therapy in various medical fields has gained the attention of the malarial biologist to make the next generation vaccines for the long-lasting immunity. Various approaches through whole sporozoites vaccines may prove to be the better vaccine candidates, however, the obstacles related to their manufacturing, formulation, and availability to mankind needs further experimental validation.

Acknowledgement

Authors would like to thank the Department of Science & Technology (DST-SERB; grant number: EMR/2014/000543) and Department of Biotechnology (DBT; grant number: BT/PR7857/BRB/10/1215/2013), Govt. of India for providing financial support to SKD, and the Council of Scientific and Industrial Research (CSIR), New Delhi, Govt. of India for providing fellowship (CSIR-SRF HRDG No.: 09/1048(0009)/2018-EMR-I) to Mr. Nikunj Tandel.

Conflict of interest

The authors declare that they have no competing interests.

Author details

Nikunj Tandel, Devang Trivedi, Aditi Mohan Krishnan and Sarat Kumar Dalai*
Institute of Science, Nirma University, Ahmedabad, Gujarat, India

*Address all correspondence to: sarat.dalai@nirmauni.ac.in

© 2020 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/3.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.
References

[1] Cox FE. History of the discovery of the malaria parasites and their vectors. Parasites and Vectors. 2010;3(1):5

[2] Tyagi RK, Tandel N, Deshpande R, Engelman RW, Patel SD, Tyagi P. Humanized mice are instrumental to the study of Plasmodium falciparum infection. Frontiers in Immunology. 2018;9:19. Article Number: 2550

[3] WHO. World malaria report 2018: World Health Organization; 2018

[4] UNICEF, Malaria Mortality Among Children Under Five is Concentrated in Sub-Saharan Africa. UNICEF Data: Monitoring the Situation of Women and Children; 2015

[5] World Health Organization. Population Mobility and Malaria. World Health Organization, Regional Office for South-East Asia. 2017. Available from: https://apps.who.int/iris/handle/10665/255816

[6] Siciliano G, Alano P. Enlightening the malaria parasite life cycle: Bioluminescent plasmodium in fundamental and applied research. Frontiers in Microbiology. 2015;6:391

[7] Cowman AF, Healer J, Marapana D, Marsh K. Malaria: Biology and disease. Cell. 2016;167(3):610-624

[8] Bertolino P, Bowen DG. Malaria and the liver: Immunological hide-and-seek or subversion of immunity from within? Frontiers in Microbiology. 2015;6:41

[9] Schleicher TR, Yang J, Freudzon M, Rembisz A, Craft S, Hamilton M, et al. A mosquito salivary gland protein partially inhibits plasmodium sporozoite cell traversal and transmission. Nature Communications. 2018;9(1):2908

[10] Prudêncio M, Rodriguez A, Mota MM. The silent path to thousands of merozoites: The plasmodium liver stage. Nature Reviews Microbiology. 2006;4(11):849

[11] Amino R, Thiberge S, Martin B, Celli S, Shorte S, Frischknecht F, et al. Quantitative imaging of plasmodium transmission from mosquito to mammal. Nature Medicine. 2006;12(2):220

[12] Sidjanski S, Vanderberg JP. Delayed migration of plasmodium sporozoites from the mosquito bite site to the blood. The American Journal of Tropical Medicine and Hygiene. 1997;57(4):426-429

[13] Yamauchi LM, Coppi A, Snounou G, Sinnis P. Plasmodium sporozoites trickle out of the injection site. Cellular Microbiology. 2007;9(5):1215-1222

[14] Guilbride DL, Guilbride PD, Gawlinski P. Malaria’s deadly secret: A skin stage. Trends in Parasitology. 2012;28(4):142-150

[15] Mota MM, Pradel G, Vanderberg JP, Hafalla JC, Frevert U, Nussenzweig RS1, et al. Migration of plasmodium sporozoites through cells before infection. Science. 2001;291(5501):141-144

[16] Ishino T, Chinzei Y, Yuda M. A plasmodium sporozoite protein with a membrane attack complex domain is required for breaching the liver sinusoidal cell layer prior to hepatocyte infection. Cellular Microbiology. 2005;7(2):199-208

[17] Ishino T, Yano K, Chinzei Y, Yuda M. Cell-passage activity is required for the malarial parasite to cross the liver sinusoidal cell layer. PLoS Biology. 2004;2(1):e4

[18] Frevert U, Engelmann S, Zougbdé S, Stange J, Ng B, Matuschewski K, et al.
Intravital observation of *Plasmodium berghei* sporozoite infection of the liver. PLoS Biology. 2005;3(6):e192

[19] Sturm A, Amino R, Van de Sand C, Regen T, Retzlaff S, Rennenberg A, et al. Manipulation of host hepatocytes by the malaria parasite for delivery into liver sinusoids. Science. 2006;313(5791):1287-1290

[20] Nilsson SK, Childs LM, Buckee C, Marti M. Targeting human transmission biology for malaria elimination. PLoS Pathogens. 2015;11(6):e1004871

[21] Weiss GE, Gilson PR, Taechalerptaisarn T, Tham WH, de Jong NW, Harvey KL, et al. Revealing the sequence and resulting cellular morphology of receptor-ligand interactions during *Plasmodium falciparum* invasion of erythrocytes. PLoS Pathogens. 2015;11(2):e1004670

[22] Baker DA. Malaria gametocytogenesis. Molecular and Biochemical Parasitology. 2010;172(2):57-65

[23] Waters AP. Epigenetic roulette in blood stream plasmodium: Gambling on sex. PLoS Pathogens. 2016;12(2):e1005353

[24] Phillips MA, Burrows JN, Manyando C, van Huisduijjn RH, Van Voorhis WC, Wells TNC. Malaria. Nature Reviews. Disease Primers. 2017;3:17050

[25] Robinson MW, Harmon C, O’Farrelly C. Liver immunology and its role in inflammation and homeostasis. Cellular and Molecular Immunology. 2016;13(3):267

[26] Trefts E, Gannon M, Wasserman DH. The liver. Current Biology. 2017;27(21):R1147-R1151

[27] Tangpukdee N, Duangdee C, Wilairatana P, Krudsood S. Malaria diagnosis: A brief review. The Korean Journal of Parasitology. 2009;47(2):93

[28] WHO. Status Report on Artemisinin and ACT Efficacy. 2018. WHO (WHO/CD/2018); 2018. p. 10

[29] WHO. Global Report on Insecticide Resistance in Malaria Vectors: 2010-2016. May 2018. p. 72. ISBN: 978-92-4-151405-7

[30] Thanh NV, Toan TQ, Cowman AF, Casey GJ, Phuc BQ, Tien NT, et al. Monitoring for *Plasmodium falciparum* drug resistance to artemisinin and artesunate in Binh Phuoc Province, Vietnam: 1998–2009. Malaria Journal. 2010;9(1):181

[31] Thanh NV, Thuy-Nhien N,uyen NTK, Tong NT, Nha-Ca NT, Quang HH, et al. Rapid decline in the susceptibility of *Plasmodium falciparum* to dihydroartemisinin–piperaquine in the south of Vietnam. Malaria Journal. 2017;16(1):27

[32] Ashley EA, Phyo AP. Drugs in development for malaria. Drugs. 2018;78(9):861-879

[33] Blasco B, Leroy D, Fidock DA. Antimalarial drug resistance: Linking *Plasmodium falciparum* parasite biology to the clinic. Nature Medicine. 2017;23(8):917

[34] Sinha S, Medhi B, Sehgal R. Challenges of drug-resistant malaria. Parasite. 2014;21:15. Article Number: 61

[35] Tyagi RK, Gleeson PJ, Arnold L, Tahar R, Prieur E, Decosterd L, et al. High-level artemisinin-resistance with quinine co-resistance emerges in *P. falciparum* malaria under in vivo artesunate pressure. BMC Medicine. 2018;16(1):181

[36] Hill AV. Vaccines against malaria. Philosophical Transactions of the Royal Society, B: Biological Sciences. 2011;366(1579):2806-2814
[37] Schofield L, Villaquiran J, Ferreira A, Schellekens H, Nussenzweig R, Nussenzweig V. γ Interferon, CD8+ T cells and antibodies required for immunity to malaria sporozoites. Nature. 1987;330(6149):664

[38] Krzych U, Dalai S, Zarling SN, Pichugin AV. Memory CD8 T cells specific for plasmodia liver-stage antigens maintain protracted protection against malaria. Frontiers in Immunology. 2012;3:370

[39] Krzych U, Schwenk R, Guebre-Xabier M, Sun P, Palmer D, White K, et al. The role of intrahepatic lymphocytes in mediating protective immunity induced by attenuated Plasmodium berghei sporozoites. Immunological Reviews. 2000;174(1):123-134

[40] Overstreet MG, Cockburn IA, Chen YC, Zavala F. Protective CD8+ T cells against plasmodium liver stages: Immunobiology of an ‘unnatural’immune response. Immunological Reviews. 2008;225(1):272-283

[41] Good MF, Doolan DL. Malaria vaccine design: Immunological considerations. Immunity. 2010;33(4):555-566

[42] Obeid M, Franetich JF, Lorthiois A, Gego A, Grüner AC, Tefit M, et al. Skin-draining lymph node priming is sufficient to induce sterile immunity against pre-erythrocytic malaria. EMBO Molecular Medicine. 2013;5(2):250-263

[43] Patel H, Yadav N, Parmar R, Patel S, Singh AP, Shrivastava N, et al. Frequent inoculations with radiation attenuated sporozoite is essential for inducing sterile protection that correlates with a threshold level of plasmodia liver-stage specific CD8+ T cells. Cellular Immunology. 2017;317:48-54

[44] Krzych U, Dalai S, Ehrler L, Jobe O, Egner L. Infectious challenge of Plasmodium berghei γ-spz immunized mice rescues effector CD8+ T cells, thus assuring protracted protection. The Journal of Immunology. 2010;184(1 Suppl):52-59

[45] Donya M, Radford M, ElGuindy A, Firmin D, Yacoub MH. Radiation in medicine: Origins, risks and aspirations. Global Cardiology Science and Practice. 2015;57:438-448

[46] Reed AB. The history of radiation use in medicine. Journal of Vascular Surgery. 2011;53(1 Suppl):3s-5s

[47] Christensen DM, Iddins CJ, Sugarman SL. Ionizing radiation injuries and illnesses. Emergency Medicine Clinics. 2014;32(1):245-265

[48] Grover S, Kumar J. A review of the current concepts of radiation measurement and its biological effects. Indian Journal of Radiology and Imaging. 2002;12(1):21-32

[49] Thomas GA, Symonds P. Radiation exposure and health effects—Is it time to reassess the real consequences? Clinical Oncology. 2016;28(4):231-236

[50] Shahbazi-Gahrouei D, Gholami M, Setayandeh S. A review on natural background radiation. Advanced biomedical research. 2013;2:11

[51] Ramachandran T. Background radiation, people and the environment. International Journal of Radiation Research. 2011;9(2):63-76

[52] Bergonie J. De quelques resultas de la radiotherapie et essai de fixation d’une technique radionnelle. Comptes rendus de l’Académie des Sciences. 1906;143:983-995

[53] Bolus NE. Basic review of radiation biology and terminology. Journal
of Nuclear Medicine Technology. 2017;45(4):259-264

[54] Muller HJ. Artificial transmutation of the gene. Science. New York, NW, Washington: American Association for the Advancement of Science (AAAS), 1200, DC 20005. 1927;66(1699):84-87

[55] Seeram E, Travis EL. Radiation Protection. Philadelphia, New York: Lippincott; 1997

[56] Dowd SB, Tilson ER. Practical Radiation Protection and Applied Radiobiology. Philadelphia & United States of America: WB Saunders Company; 1999

[57] Authors of ICRP, Stewart FA, Akleyev AV, Hauer-Jensen M, Hendry JH, Kleiman NJ, et al. ICRP publication 118: ICRP statement on tissue reactions and early and late effects of radiation in normal tissues and organs–threshold doses for tissue reactions in a radiation protection context. Annals of the ICRP. 2012;41(1-2):1-333

[58] Seo HS. Application of radiation technology in vaccines development. Clinical and Experimental Vaccine Research. 2015;4(2):145-158

[59] Hendry J. Radiation biology and radiation protection. Annals of the ICRP. 2012;41(3-4):64-71

[60] Baskar R, Lee KA, Yeo R, Yeoh K-W. Cancer and radiation therapy: Current advances and future directions. International Journal of Medical Sciences. 2012;9(3):193

[61] Cadena A, Cushman T, Anderson C, Barsoumian H, Welsh J, Cortez M. Radiation and anti-cancer vaccines: A winning combination. Vaccine. 2018;6(1):9

[62] Garnett-Benson C, Hodge JW, Gameiro SR. Combination regimens of radiation therapy and therapeutic cancer vaccines: Mechanisms and opportunities. Seminars in Radiation Oncology. 2015;25(1):46-53

[63] Citrin DE. Recent developments in radiotherapy. New England Journal of Medicine. 2017;377(11):1065-1075

[64] Janiak MK, Wencenciak M, Cheda A, Nowosielska EM, Calabrese EJ. Cancer immunotherapy: How low-level ionizing radiation can play a key role. Cancer Immunology, Immunotherapy. 2017;66(7):819-832

[65] Qin L, Smith BD, Tsai H, Yaghi N, Neela P, Moake M, et al. Induction of high-titer IgG antibodies against multiple leukemia-associated antigens in CML patients with clinical responses to K562/GVAX immunotherapy. Blood Cancer Journal. 2013;3(9):e145

[66] De Remigis A, De Gruijil TD, Uram JN, Tzou SC, Iwama S, Talor MV, et al. Development of thyroglobulin antibodies after GVAX immunotherapy is associated with prolonged survival. International Journal of Cancer. 2015;136(1):127-137

[67] Le DT, Wang-Gillam A, Picozzi V, Greten TF, Crocenzi T, Springett G, et al. Safety and survival with GVAX pancreas prime and listeria monocytogenes–expressing mesothelin (CRS-207) boost vaccines for metastatic pancreatic cancer. Journal of Clinical Oncology. 2015;33(12):1325

[68] Li Y, Wang Z, Liu X, Tang J, Peng B, Wei Y. X-ray irradiated vaccine confers protection against pneumonia caused by Pseudomonas aeruginosa. Scientific Reports. 2016;6:18823

[69] Arama C, Troye-Blomberg M. The path of malaria vaccine development: Challenges and perspectives. Journal of Internal Medicine. 2014;275(5):456-466

[70] Jindal H, Bhatt B, Malik JS, Mehta B. Malaria vaccine: A step
toward elimination. Human Vaccines and Immunotherapeutics. 2014;10(6):1752-1754

[71] Beeson JG, Kurtovic L, Dobaño C, Opi DH, Chan JA, Feng G, et al. Challenges and strategies for developing efficacious and long-lasting malaria vaccines. Science Translational Medicine. 2019;11(474):eaau1458

[72] Mahmoudi S, Keshavarz H. Malaria vaccine development: The need for novel approaches: A review article. Iranian Journal of Parasitology. 2018;13(1):1

[73] Deitsch KW, Dzikowski R. Variant gene expression and antigenic variation by malaria parasites. Annual Review of Microbiology. 2017;71:625-641

[74] Rénia L, Goh YS. Malaria parasites: The great escape. Frontiers in Immunology. 2016;7:463

[75] Belachew EB. Immune response and evasion mechanisms of *Plasmodium falciparum* parasites. Journal of Immunology Research. 25 Mar 2018;2018:6529681

[76] Doolan DL. Plasmodium immunomics. International Journal for Parasitology. 2011;41(1):3-20

[77] Kooij TW, Janse CJ, Waters AP. Plasmodium post-genomics: Better the bug you know? Nature Reviews Microbiology. 2006;4(5):344

[78] Vaughan AM, Kappe SH. Malaria vaccine development: Persistent challenges. Current Opinion in Immunology. 2012;24(3):324-331

[79] Draper SJ, Sack BK, King CR, Nielsen CM, Rayner JC, Higgins MK, et al. Malaria vaccines: Recent advances and new horizons. Cell Host and Microbe. 2018;24(1):43-56

[80] Coelho CH, Doritchamou JYA, Zaidi I, Duffy PE. Advances in malaria vaccine development: Report from the 2017 malaria vaccine symposium. NPJ Vaccines. 2017;2:34

[81] Vaughan AM, Aly AS, Kappe SH. Malaria parasite pre-erythrocytic stage infection: Gliding and hiding. Cell Host and Microbe. 2008;4(3):209-218

[82] Nussenzweig R, Vanderberg J, Most H, Orton C. Protective immunity produced by the injection of x-irradiated sporozoites of *Plasmodium berghei*. Nature. 1967;216(5111):160

[83] Nussenzweig V, Nussenzweig RS. Development of a sporozoite malaria vaccine. The American Journal of Tropical Medicine and Hygiene. 1986;35(4):678-688

[84] Dame JB, Williams JL, McCutchan TF, Weber JL, Wirtz RA, Hockmeyer WT, et al. Structure of the gene encoding the immunodominant surface antigen on the sporozoite of the human malaria parasite *Plasmodium falciparum*. Science. 1984;225(4662):593-599

[85] Gordon D, McGovern T, Krzych U, Cohen J, Schneider I, LaChance R, et al. Safety, immunogenicity, and efficacy of a recombinantly produced *Plasmodium falciparum* circumsporozoite protein-hepatitis B surface antigen subunit vaccine. Journal of Infectious Diseases. 1995;171(6):1576-1585

[86] Kester KE, Cummings JF, Ofori-Anyinam O, Ockenhouse CF, Krzych U, Moris P, et al. Randomized, double-blind, phase 2a trial of falciparum malaria vaccines RTS, S/AS01B and RTS, S/AS02A in malaria-naive adults: Safety, efficacy, and immunologic associates of protection. Journal of Infectious Diseases. 2009;200(3):337-346

[87] Mahmoudi S, Keshavarz H. Efficacy of phase 3 trial of RTS, S/AS01 malaria vaccine: The need for an
alternative development plan. Human Vaccines and Immunotherapeutics. 2017;13(9):2098-2101

[88] Crompton PD, Pierce SK, Miller LH. Advances and challenges in malaria vaccine development. The Journal of Clinical Investigation. 2010;120(12):4168-4178

[89] Sagara I, Dicko A, Ellis RD, Fay MP, Diawara SI, Assadou MH, et al. A randomized controlled phase 2 trial of the blood stage AMA1-C1/Alhydrogel malaria vaccine in children in Mali. Vaccine. 2009;27(23):3090-3098

[90] El Sahly H, Patel S, Atmar R, Lanford T, Dube T, Thompson D, et al. The safety and immunogenicity of recombinant EBA 175-RII NG malaria vaccine in healthy adults living in a non-endemic area. Clinical and Vaccine Immunology. 2010;17(10):1552-1559

[91] Esen M, Kremsner PG, Schleucher R, Gassler M, Imoukhuede EB, Imbault N, et al. Safety and immunogenicity of GMZ2—A MSP3–GLURP fusion protein malaria vaccine candidate. Vaccine. 2009;27(49):6862-6868

[92] Hermen CC, Verhage DF, Telgta DS, Teelen K, Bouwema JT, Roestenberg M, et al. Glutamate-rich protein (GLURP) induces antibodies that inhibit in vitro growth of Plasmodium falciparum in a phase 1 malaria vaccine trial. Vaccine. 2007;25(15):2930-2940

[93] Ogutu BR, Apollo OJ, McKinney D, Okoth W, Siangla J, Dubovsky F, et al. Blood stage malaria vaccine eliciting high antigen-specific antibody concentrations confers no protection to young children in Western Kenya. PLoS One. 2009;4(3):e4708

[94] Gorton B, Betuela I, Felger I, Al-Yaman F, Anders RF, Saul A, et al. A recombinant blood-stage malaria vaccine reduces Plasmodium falciparum density and exerts selective pressure on parasite populations in a phase 1-2b trial in Papua New Guinea. The Journal of Infectious Diseases. 2002;185(6):820-827

[95] Audran R, Cachat M, Lurati F, Soe S, Leroy O, Corradin G, et al. Phase I malaria vaccine trial with a long synthetic peptide derived from the merozoite surface protein 3 antigen. Infection and Immunity. 2005;73(12):8017-8026

[96] Sirima SB, Tiono AB, Ouédraogo A, Diarra A, Ouédraogo AL, Yaro JB, et al. Safety and immunogenicity of the malaria vaccine candidate MSP3 long synthetic peptide in 12-24 months-old Burkinabe children. PLoS One. 2009;4(10):e7549

[97] Druilhe P, Spertini F, Soesoe D, Corradin G, Mejia P, Singh S, et al. A malaria vaccine that elicits in humans antibodies able to kill Plasmodium falciparum. PLoS Medicine. 2005;2(11):e344

[98] Horii T, Shirai H, Jie L, Ishii KJ, Palacpac NQ, Tougan T, et al. Evidences of protection against blood-stage infection of Plasmodium falciparum by the novel protein vaccine SE36. Parasitology International. 2010;59(3):380-386

[99] Miura K. Progress and prospects for blood-stage malaria vaccines. Expert Review of Vaccines. 2016;15(6):765-781

[100] Carter R, Mendis KN, Miller LH, Molineaux L, Saul A. Malaria transmission-blocking vaccines—How can their development be supported? Nature Medicine. 2000;6(3):241

[101] Graves PM, Carters R, Burkot TR, Quakyi IA, Kumar N. Antibodies to Plasmodium falciparum gamete surface antigens in Papua New Guinea sera. Parasite Immunology. 1988;10(2):209-218

[102] Bousema J, Drakeley C, Sauerwein R. Sexual-stage antibody responses to
Application of Radiation Technology: A Novel Vaccine Approach to Induce Protective Immunity...
DOI: http://dx.doi.org/10.5772/intechopen.85491

P. falciparum in endemic populations. Current Molecular Medicine. 2006;6(2):223-229

[103] Scaria PV, Chen B, Rowe CG, Jones DS, Barnafo E, Fischer ER, et al. Protein-protein conjugate nanoparticles for malaria antigen delivery and enhanced immunogenicity. PLoS One. 2017;12(12):e0190312

[104] Russell PF, Mohan B. The immunization of fowls against mosquito-borne Plasmodium gallinaceum by injections of serum and of inactivated homologous sporozoites. Journal of Experimental Medicine. 1942;76(5):477-495

[105] Hoffman SL, Goh LM, Luke TC, Schneider J, Le TP, Doolan DL, et al. Protection of humans against malaria by immunization with radiation-attenuated Plasmodium falciparum sporozoites. The Journal of Infectious Diseases. 2002;185(8):1155-1164

[106] Roestenberg M, McCall M, Hopman J, Wiersma J, Luty AJ, van Gemert GJ, et al. Protection against malaria after experimental sporozoite inoculation: An open-label follow-up study. The Lancet. 2011;377(9779):1770-1776

[107] Roestenberg M, Teirlinck AC, McCall MB, Teelen K, Makamdop KN, Wiersma J, et al. Long-term protection against malaria after experimental sporozoite inoculation: An open-label follow-up study. The Lancet. 2011;377(9779):1770-1776

[108] Laurens MB, Billingsley P, Richman A, Eappen AG, Adams M, Li T, et al. Successful human infection with P. falciparum using three aseptic Anopheles stephensi mosquitoes: A new model for controlled human malaria infection. PLoS One. 2013;8(7):e68969

[109] Seder RA, Chang L-J, Enama ME, Zephir KL, Sarwar UN, Gordon IJ, et al. Protection against malaria by intravenous immunization with a nonreplicating sporozoite vaccine. Science. 2013;341(6152):1359-1365

[110] Epstein JE, Richie TL. The whole parasite, pre-erythrocytic stage approach to malaria vaccine development: A review. Current Opinion in Infectious Diseases. 2013;26(5):420-428

[111] Hoffman SL, Billingsley PF, James E, Richman A, Loyevsky M, Li T, et al. Development of a metabolically active, non-replicating sporozoite vaccine to prevent Plasmodium falciparum malaria. Human Vaccines. 2010;6(1):97-106

[112] Epstein JE, Tewari K, Lyke K, Sim B, Billingsley P, Laurens M, et al. Live attenuated malaria vaccine designed to protect through hepatic CD8+ T cell immunity. Science. 2011;334(6055):475-480

[113] Luke TC, Hoffman SL. Rationale and plans for developing a non-replicating, metabolically active, radiation-attenuated Plasmodium falciparum sporozoite vaccine. Journal of Experimental Biology. 2003;206(21):3803-3808

[114] Richie TL, Billingsley PF, Sim BKL, James ER, Chakravarty S, Epstein JE, et al. Progress with Plasmodium falciparum sporozoite (PfSPZ)-based malaria vaccines. Vaccine. 2015;33(52):7452-7461

[115] Malik A, Egan JE, Houghten RA, Sadoff JC, Hoffman SL. Human cytotoxic T lymphocytes against the Plasmodium falciparum circumsporozoite protein. Proceedings of the National Academy of Sciences. 1991;88(8):3300-3304

[116] Wizel B, Houghten R, Church P, Tine JA, Lanar DE, Gordon DM, et al. HLA-A2-restricted cytotoxic T lymphocyte responses to multiple Plasmodium falciparum sporozoite
surface protein 2 epitopes in sporozoite-immunized volunteers. The Journal of Immunology. 1995;155(2):766-775

[117] Krzych U, Lyon JA, Jareed T, Schneider I, Hollingdale MR, Gordon DM, et al. T lymphocytes from volunteers immunized with irradiated Plasmodium falciparum sporozoites recognize liver and blood stage malaria antigens. The Journal of Immunology. 1995;155(8):4072-4077

[118] Nardin EH, Herrington DA, Davis J, Levine M, Stuber D, Takacs B, et al. Conserved repetitive epitope recognized by CD4+ clones from a malaria-immunized volunteer. Science. 1989;246(4937):1603-1606

[119] Gwadz R, Cochrane A, Nussenzweig V, Nussenzweig R. Preliminary studies on vaccination of rhesus monkeys with irradiated sporozoites of Plasmodium knowlesi and characterization of surface antigens of these parasites. Bulletin of the World Health Organization. 1979;57(Suppl):165-173

[120] Egan JE, Hoffman SL, Haynes JD, Sadoff JC, Schneider I, Grau GE, et al. Humoral immune responses in volunteers immunized with irradiated Plasmodium falciparum sporozoites. The American Journal of Tropical Medicine and Hygiene. 1993;49(2):166-173

[121] Zavala F, Tam JP, Hollingdale MR, Cochrane AH, Quakyi I, Nussenzweig RS, et al. Rationale for development of a synthetic vaccine against Plasmodium falciparum malaria. Science. 1985;228(4706):1436-1440

[122] Ballou WR, Sherwood JA, Neva FA, Gordon DM, Wirtz RA, Wasserman GF, et al. Safety and efficacy of a recombinant DNA Plasmodium falciparum sporozoite vaccine. Bethesda, MD: Naval Medical Research Institute; 1987

[123] Olotu A, Fegan G, Wambua J, Nyangweso G, Awuondo KO, Leach A, et al. Four-year efficacy of RTS, S/AS01E and its interaction with malaria exposure. New England Journal of Medicine. 2013;368(12):1111-1120

[124] White MT, Bejon P, Olotu A, Griffin JT, Bojang K, Lusingu J, et al. A combined analysis of immunogenicity, antibody kinetics and vaccine efficacy from phase 2 trials of the RTS, S malaria vaccine. BMC Medicine. 2014;12(1):117

[125] Moorthy VS, Ballou WR. Immunological mechanisms underlying protection mediated by RTS, S: A review of the available data. Malaria Journal. 2009;8(1):312

[126] Sissoko MS, Healy SA, Katile A, Omaswa F, Zaidi I, Gabriel EE, et al. Safety and efficacy of PfSPZ vaccine against Plasmodium falciparum via direct venous inoculation in healthy malaria-exposed adults in Mali: A randomised, double-blind phase 1 trial. The Lancet Infectious Diseases. 2017;17(5):498-509

[127] Sinden R. The cell biology of malaria infection of mosquito: Advances and opportunities. Cellular Microbiology. 2015;17(4):451-466

[128] Karanja J, Kiboi N. Current milestones towards development of a fully deployable anti-malaria vaccine-future hope for malaria-free world: A review. Journal of Vaccines and Vaccination. 2016;7(332):2