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Malaria, an ancient disease has been found recorded in several historical documents and the word malaria comes from the Italian mal'aria meaning spoiled air [1].

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

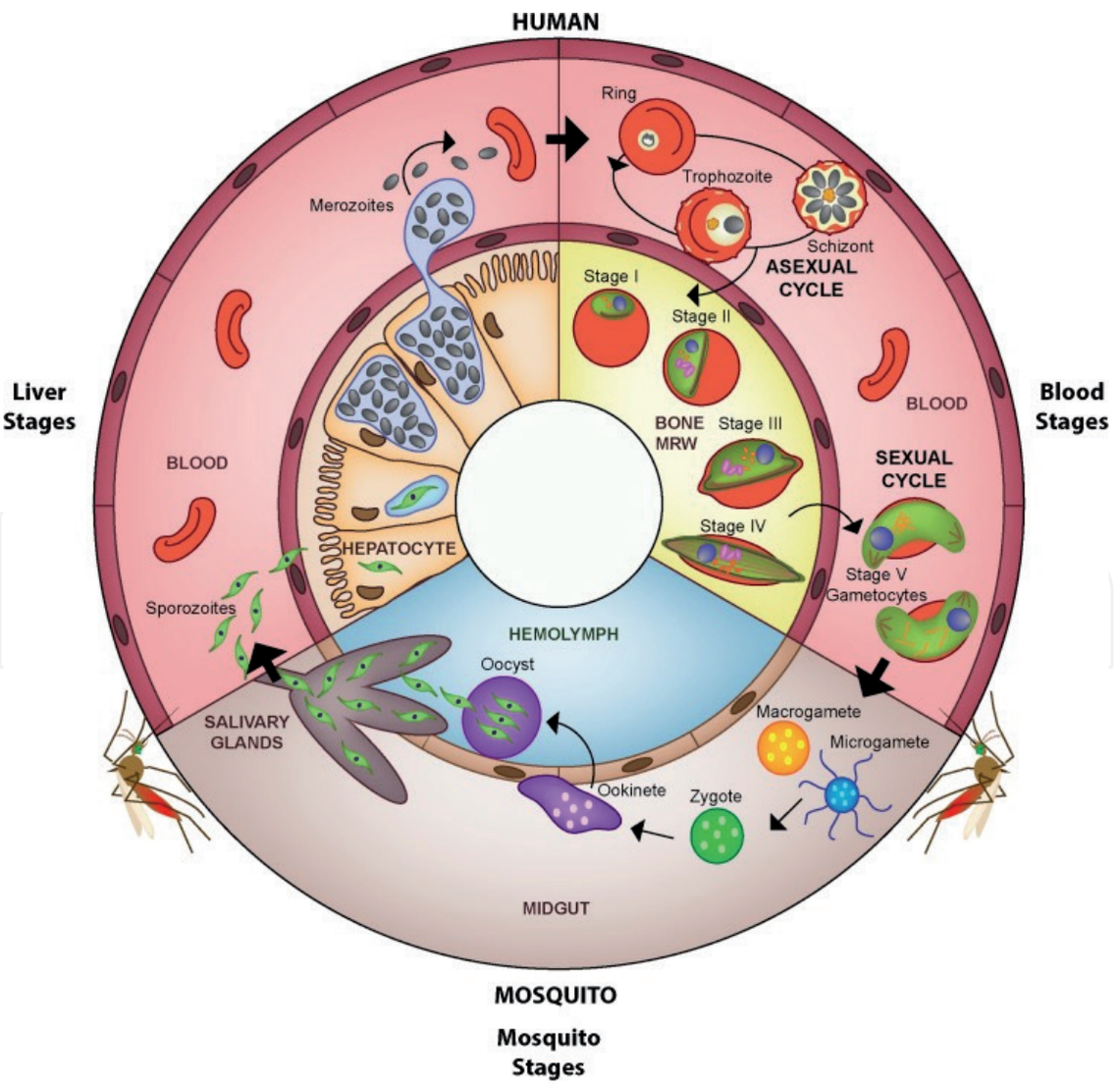


Figure 1.
The basic malaria life cycle of Plasmodium falciparum (adapted from [20] with permission).

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**).

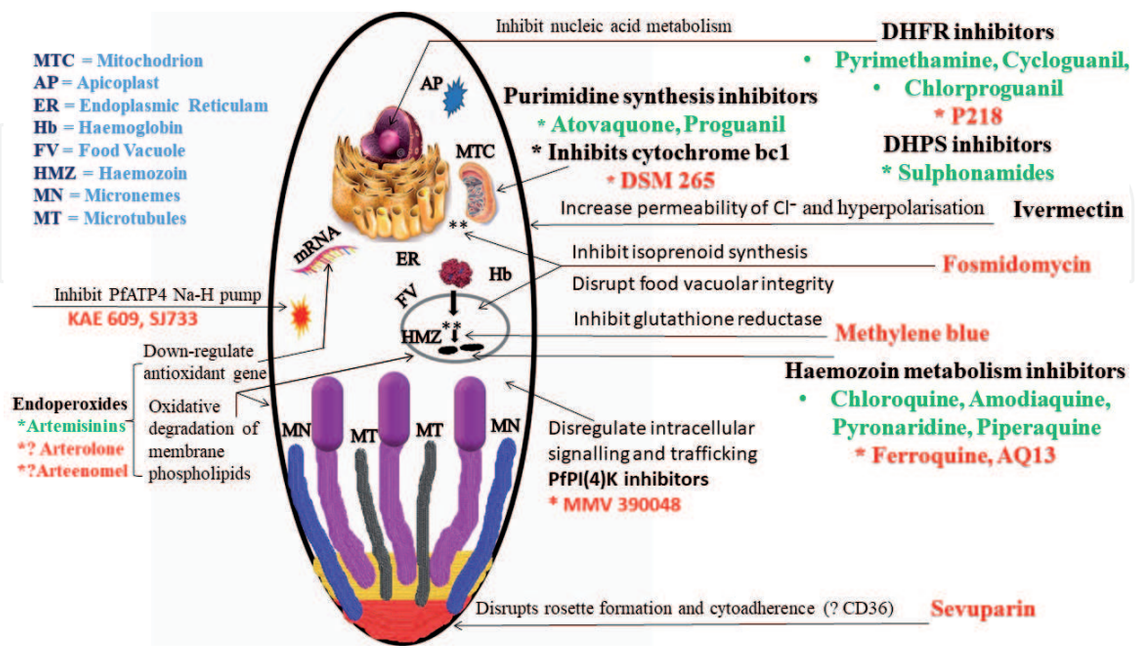


Figure 2.
Schematic diagram of intra-erythrocytic trophozoite showing targets of novel antimalarial drugs (red color indicates the drugs under the pipeline/clinical trials and green color for the drugs currently available) (adapted and modified from [32]).

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 adjutants, 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 CD 8+ 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

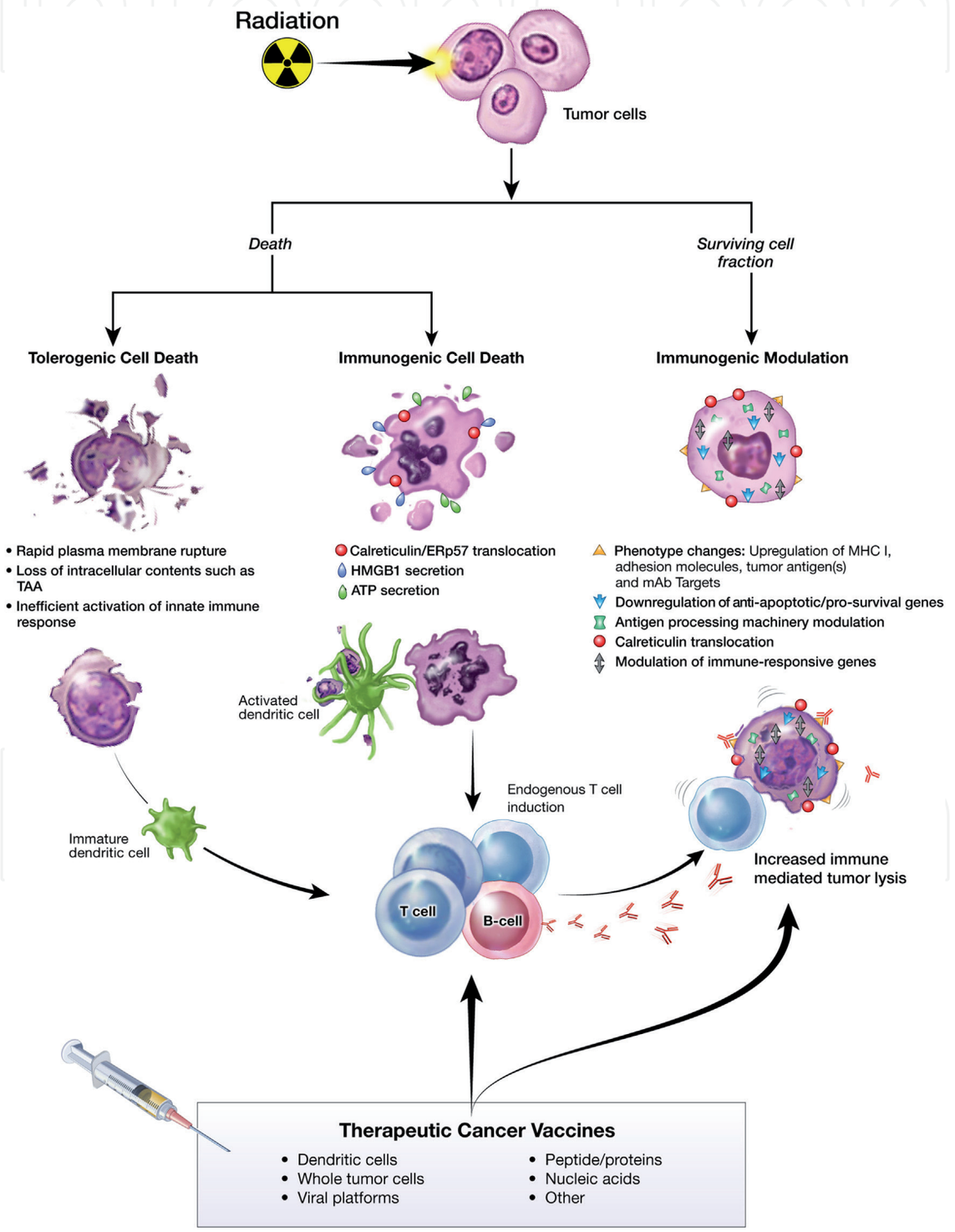


Figure 3.
Radiation therapy and their different consequences can be used for antitumor combination therapy for cancer therapeutics (adapted from [62] with permission).

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 Pfs25 and Pfs28 and their *P. vivax* homologs Pvs25 and Pvs28. To improve the immunogenicity, Pfs25 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 placebos) [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 *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 *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.

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Conflict of interest

The authors declare that they have no competing interests.

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