

Current Milestones towards Development of a Fully Deployable Anti-Malaria Vaccine-Future Hope for Malaria-Free World: A Review

Karanja JK^{1*} and Kiboi NG²

¹Department of Zoological Sciences, School of Pure and Applied Sciences, Kenyatta University, P.O. Box: 43844-00100, Nairobi, Kenya

²Department of Biochemistry and Biotechnology, School of Pure and Applied Sciences, Kenyatta University, P.O. BOX: 43844-00100, Nairobi, Kenya

*Correspondence author: Karanja JK, Department of Zoological Sciences, School of Pure and Applied Sciences, Kenyatta University, Nairobi, Kenya, Tel: +254726721424; E-mail: jkaranja43@yahoo.com

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Abstract

Despite novel global measures to combat malaria, the disease remains a considerable healthcare burden especially in resource limited settings. It accounts for over 2 million deaths per year, most of which are among young children and pregnant women. Despite intensive research and development, only one candidate vaccine, radiation-attenuated sporozoite (RAS, S) has made considerable progress to phase 3 clinical trials, albeit a documented partial efficacy of 46% against clinical malaria. However, it's on the road map of becoming the first licensed malaria vaccine with identified potential for development of deployable malaria vaccine. Success of this candidate forms a vital public health tool designed to eradicate global malaria. Parasite antigenic diversity, poor understanding of anti-malarial immunity, and lack of immune correlates of protection constitute among the major hindrances of developing an effective malaria vaccine. Current vaccine models such as RAS, S targets *Plasmodium falciparum* during the pre-erythrocytic and erythrocytic stages, while a few other interventions direct their activity by blocking transmission against asexual stages, and/or against pregnancy-associated malaria. Recombinant vaccines have initially been designed from antigens containing one or two strains, which represents a significantly small fraction of the genetic diversity of malaria parasites, eventually making it cumbersome for investigators to establish strain-specific efficacy in clinical trials. This current review, therefore, seeks to provide an overview of major achievements in malaria research; highlighting potential applications, confounders while also showcasing future directions that purpose to enhance discovery of safe and effective anti-malaria vaccines.

Keywords: Malaria; Vaccine; Clinical trials; *Plasmodium falciparum*; Immunity

Abbreviations:

RAS: Radiation Attenuated Sporozoite; *pf*: *Plasmodium falciparum*; *p. vivax*: *Plasmodium vivax*; pb GAP: *Plasmodium bergeri* Genetically Attenuated parasites; WHO: World Health Organization; CSP: Circumsporozoite Protein; pfEMP1: *Plasmodium falciparum* erythrocyte membrane protein 1; CSA: Chondroitin Surface Antigen.

Introduction

Malaria ranks high among the most devastating parasitic diseases afflicting humankind. The disease is caused by parasites of the genus *plasmodium*, and is transmitted by female anopheles mosquitoes [1]. *Plasmodium falciparum* is the most virulent of the five known human malaria parasites accounting for 90% of malaria-related deaths globally [2]. The parasite is endemic within Sub-Saharan region of Africa [3,4]. On the other hand, infections occurring outside this region are mainly attributable to *P. vivax* which is less deadly compared *P. falciparum* [5]. Interestingly, *P. vivax* remains dormant in the human liver, a unique feature in its biology that accounts for its challenging elimination; which hampers its elimination in endemic countries [6]. Thus, malaria continues to exert robust health toll and economic burden globally, more so in resource-constrained settings [1].

Standard immunization based on *plasmodium* whole-organism and radiation-attenuated sporozoite (RAS), was assessed in mice, primates,

and humans over three decades ago, but became untenable as it required either several bites of irradiated mosquitoes or intravenous inoculation of sporozoites, both of which were considered impractical for mass vaccination [7,8]. Likewise, research on Subunit protein or DNA- based vaccines has not yielded desirable results with respect to malaria vaccine efficacy trials. Consequently, this has triggered scaling up of *P. falciparum* RAS in order optimize mass vaccination using this attenuated whole-organism approach. For instance, *P. bergeri* genetically attenuated parasites (Pb GAP) and *P. yoelii* GAP are observed to confer similar protection in experimental mice models [7,9,10].

Ultimately, among the biggest drawbacks of developing effective malaria vaccine would be the hardship of differentiating between the diversity associated with immune escape as well as cross protection. Nonetheless, polymorphisms on malaria antigens targeted by functional antibodies and how they mediate vaccine escape in plasmodium species still remain largely undefined at present [11,12]. Breakthrough strategies are dependent on the identification of immunologically relevant diversity through population genetics and structural studies that identify functional polymorphisms. In addition, epidemiological surveys control the polymorphisms to be considered when developing a multivalent malaria vaccine for multiple strains [13]. Overall, although considerable strides have been made based on different approaches to come up with an effective malaria vaccine, quest for the valuable tool remain unfulfilled.

Lifecycle of Malaria

Malaria is caused by a unicellular eukaryotic parasite of the genus plasmodium; 5 species including *P. falciparum*, *P. vivax*, *P. ovale*, *P. knowlesi* and *P. malariae* are infectious to humankind. Parasitic forms in sporozoite stage are transmitted to human host through a bite from a female anopheles mosquito [14]. Sporozoites find their way into the hepatocytes where they multiply into their progeny within 6 days, and collectively form the pre-erythrocytic parasites. Once in the liver, development continues in the hepatocytes with each sporozoite producing a schizont that progressively matures to release thousands of merozoites into the venous circulation [15-17].

The infected erythrocytes rupture, triggering onset of clinical symptoms including fever, headache, chills, and malaise; upon which

severity varies with parasite load [14]. Merozoites continually invade new erythrocytes and the cycle continues. Other factors such as host immune status, general health and nutritional conditions affect the severity of human malaria infection [18]. Uncomplicated malaria is more common in adolescents and adults in high transmission areas, whereas severe malaria, which mostly results from *P. falciparum* infections, is frequently observed among young children and adults who travel to areas of high malaria transmission. In extreme cases where the infected red blood cells are sequestered in the brain, cerebral malaria may occur with convulsions, followed by coma, and eventual death [18].

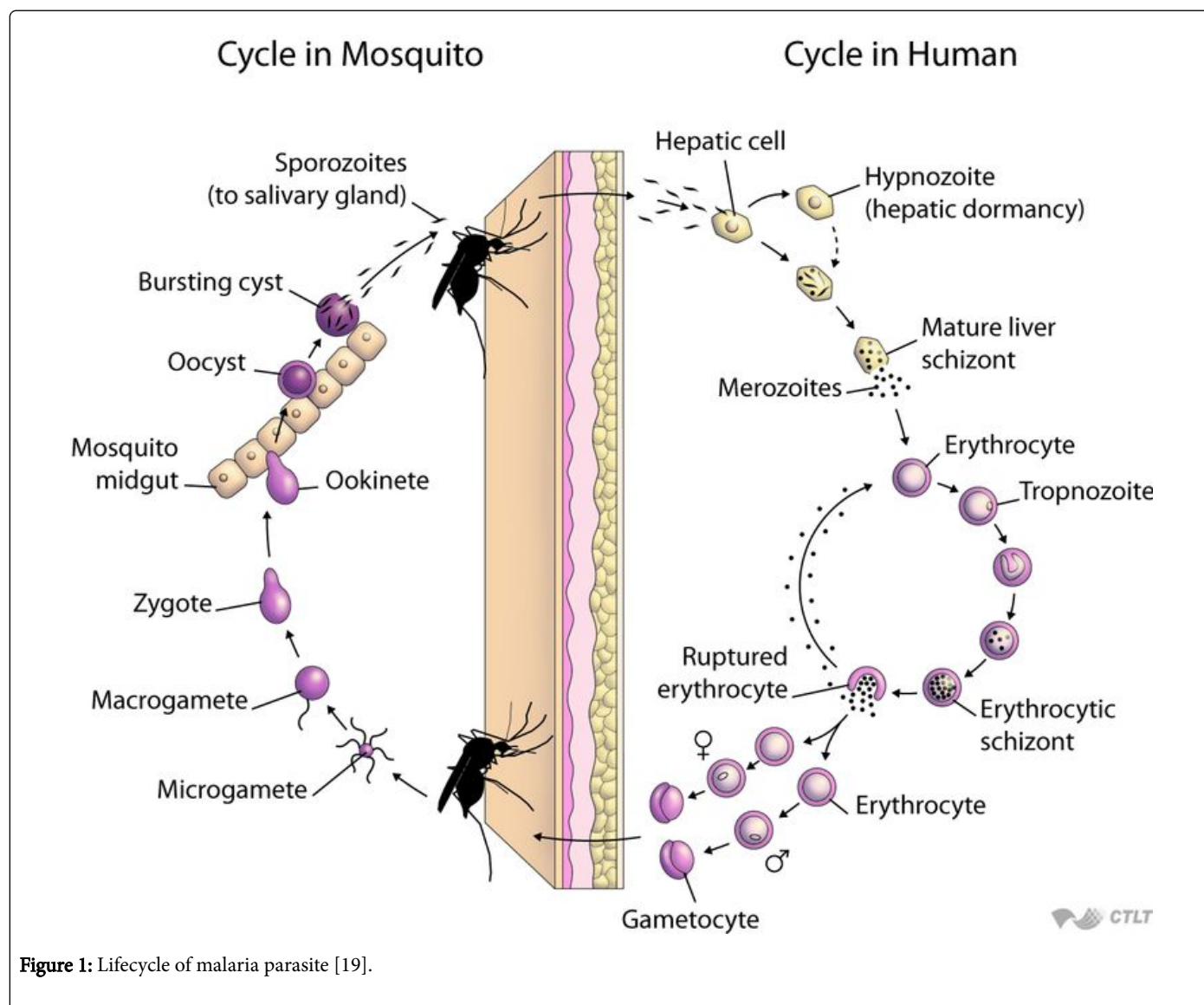


Figure 1: Lifecycle of malaria parasite [19].

Current Advances towards Malaria Vaccine Development

Over the last decade, rigorous attempts have been made towards development of malaria vaccine, and notable progress has been realized in the identification and evaluation of malaria vaccine

candidates. The first major advancement geared towards this goal began with the delivery of irradiated *P. falciparum* sporozoites intravenously, a noble discovery that triggered criticism especially amongst researchers having been considered impractical for mass vaccination [8]. Yet in 2006, WHO set a landmark objective to develop and license an efficacious malaria vaccine that confers greater than

50% protection over a period lasting one year and above by 2015 [20]. So far, only one candidate vaccine RTS, S/AS02A is on course towards realization of this goal, having demonstrated 46% efficacy. Activity of this potential vaccine is based on repeat regions and T-cell epitopes of circumsporozoite protein (CSP), and is a key focus of many phase 3 clinical trials among children in sub-Saharan Africa [21]. However, the vaccine only confers partial protection and diminishes over time [22], though its licensing may prove to be imperative in the current malaria control. All in all, more effective second-generation vaccines that would overcome the aforementioned challenges are urgently required.

Morphologically, malaria parasites are complex with many antigenic targets [23]. Regardless of this biological adaptation, the genetically and chemically-attenuated sporozoite vaccinations have shown relatively promising results in malaria clinical trials [24,25]. However, they are not without their fair share of challenges, hence an alternative approach based on subunit vaccines has been adopted [26]. Subunit

vaccines constitute individual recombinant parasite proteins administered either as monovalent or combined preparations incorporating other vectors and adjuvants in order to heighten immune responses [27-29]. They therefore have been categorized into three groups depending on the specific stage targeted in the lifecycle.

The *pre-erythrocytic vaccines* including RTS, S usually target the infective sporozoite stage to prevent infection. In addition, they also focus on antigens expressed by parasites at liver stage, by hindering release of merozoites into the bloodstream which are responsible for symptomatic malaria [30]. The major challenge associated with antigen targeting is that antigenic dose released during natural infection by the vector is quite low to elicit an effective immune response. Furthermore, it only requires a single sporozoite to escape vaccine-mediated immune responses and invade liver cells to produce thousands of merozoites in the blood stream culminating to clinical disease [30].

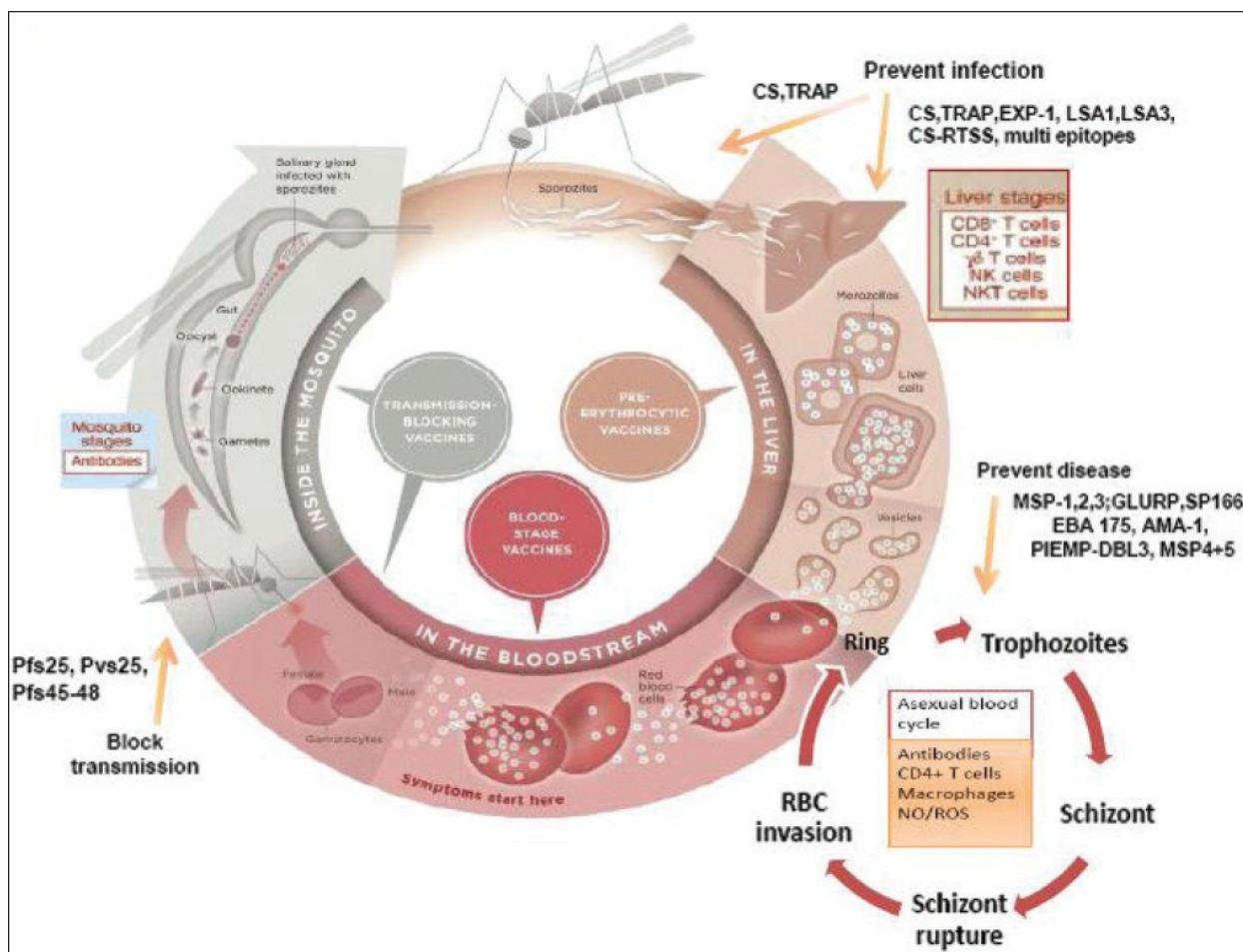


Figure 2: Illustration of malarial parasite lifecycle showing approaches of vaccine development and examples of vaccines. A modification from the Editorial commentary by Breman JG, JID 2009: 200, 317-320 (1st August) (Program for appropriate technology in health- malaria vaccine initiative) [41].

On the other hand, the *Blood stage vaccines* form majority of candidate vaccines and as the name denotes they control infection at the blood stage sub-level. They target merozoite antigens to prevent red blood cell invasion, thereby reducing the density and prevalence of

parasites in the infected host [31]. A Reduction in parasite density has been revealed to minimize density of transmission forms i.e. gametocytes (sexual stage of parasite transmitted from human to host mosquito) [32]. More novel approaches targeting major surface

proteins expressed on *P. falciparum* infected red blood cell (erythrocyte membrane protein 1, PfEMP1) are underway. However, the PfEMP1 mediates cyto-adherence to host cells and is associated with severe malaria [33].

The *Transmission blocking vaccines* comprise another group within subunit vaccines that principally target gametocyte or oocyst antigens expressed in the life stages of mosquito host [34,35]. Such vaccines are not directly involved in combating clinical disease but contribute enormously towards elimination efforts by hindering transmission [32]. Yet another class of subunit vaccines targets “*pregnancy-associated malaria*” which occurs when infected erythrocytes sequester on placental endothelial cells, a process mediated by interaction of PfEMP1 and receptors on the placental matrix, especially, chondroitin sulfate antigen (CSA) [36,37]. This binding reduces blood supply to the placenta resulting in low birth weight or pre-term delivery [38]. This condition selectively affects women who have not previously developed an immune response to PfEMP1 proteins that confer protection to subsequent pregnancies [39]. Durable B cell immunity to PfEMP1 proteins is the target for candidate vaccine that is currently in pre-clinical stage, targeting pre-reproductive age females [40].

Barriers underlying development of fully deployable malaria vaccine

Designing an effective malaria vaccine remains a demanding scientific and financial venture, owing to the complex nature of *plasmodium* with multi-stage life cycle stages, during which the parasite produces multiple antigens with different variability [42]. Additionally, yet another major hindrance to vaccine development lies in the choice of antigen (s) to be selected for inclusion [20]. To add further, the majority of approaches in this scope of investigation have been based on imperfect animal model in vitro assays with remarkable limitations, and also sero-epidemiological assessments of naturally acquired immunity against malaria in humans [43]. Most importantly, surrogate measures of protection for malaria are yet to be identified. At present, an estimated 100 malaria candidate vaccines are in development based on few antigens of *P. falciparum*, with only a limited number entering the clinical phase studies whereas the majority lie within the pre-clinical phases [44].

On the other hand, extensive diversity of potential candidate antigens has not been properly exploited in the quest to develop effective malaria vaccines. Majority of the subunit vaccine candidates in clinical trials are monovalent [13,45]. In addition, these candidate vaccines are based on a few parasite isolates such as *3D7*, *FC27*, *FUP*, *FVO* for *P. falciparum*, and *sal.1* for *P. vivax*, which poorly represent variant parasite strains in natural population [18,46]. Taken together, these phenomena explain why many candidate vaccines do not achieve the desired clinical efficacy [2,47]. Nonetheless, multivalent candidate vaccine with multiple serotypes would be more effective against a wide range of parasite strains. However, major setbacks behind this noble approach lie in diverse polymorphisms of serotypes, and alleles to be included in vaccine development [1,48]. Previous research findings, have therefore erased doubts that variable efficacy of most malaria vaccines is due to parasite genetic diversity and the strain-specific immunity to diverse parasite antigens [29,49,50].

Conclusion and Future Perspectives

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. It is evident from previous malaria elimination attempts that, for this valued goal to be achieved, malaria transmission must be thwarted. Therefore, intervention measures that focus on reducing parasite reservoir, and limit the rate at which infections spread call for our urgent consideration.

Author Contributions

Karanja JK and Kiboi NG compiled information, drafted and reviewed the manuscript.

Conflict of Interest Statement

The authors declare that the research was conducted without any issues that could be perceived as a potential conflict of interest.

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